Biosystematics and taxonomy of the *Ozothamnus leptophyllus* (Compositae) complex in New Zealand

---

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Doctor of Philosophy

in the

University of Canterbury

by I. Schönberger

University of Canterbury

2002

PUBLICATION OF NAMES

This thesis is not intended by the author to be the place of publication of names of taxa. These are merely proposed in anticipation of later publication.

Frontispiece *Cassinia leptophylla* (G.Forst.) R.Br. Plate number 484 (Part XXIII New Zealand) of the Banks' Florilegium (1980-1990).

Abstract

Historically, *Ozothamnus* in New Zealand has been separated into five endemic species and seven varieties, and more recently united into a single, undivided, but polymorphic species. However, investigation leading to the recognition of a single species was limited and many botanists continue to recognise different forms within this species based on appearance and distribution. The conflicting taxonomic opinions regarding *O. leptophyllus* are influenced by complex patterns of variation, mostly quantitative taxonomic characters, and wide distribution with overlapping ranges. In order to resolve the taxonomy of *O. leptophyllus*, morphological and genetic diversity within and among populations are assessed from throughout New Zealand and a comprehensive revision is undertaken.

Eighty morphological characters are assessed for 192 OTUs from 90 populations, covering the distribution and observed variation of *O. leptophyllus* throughout New Zealand. Agglomerative clustering methods and principal coordinate analysis are used to determine groups within the morphological data sets. Amplified fragment length polymorphism (AFLP) is used to examine the genetic diversity within *O. leptophyllus*. The genetic diversity between populations is assessed based on single individuals from 23 populations throughout New Zealand, while 12 individuals from a single homogeneous population are used to assess intra-population variation.

Strong congruence is found between the results of morphological and AFLP analyses, indicating that the morphological patterns of variation have a genetic basis. The resulting groupings support the presence of distinct entities showing variation at a similar level. These entities are recognised here as six subspecies. One subspecies can be further divided into subgroups, recognised here as three varieties. Descriptions of the taxa, typification, and a dichotomous key are provided to facilitate their identification.

Table of Contents

List of Figures

List of Tables

Chapter 1

General Introduction

This research focuses on the *Ozothamnus leptophyllus* complex, which is endemic to New Zealand. Since its first discovery more than two centuries ago *O. leptophyllus* has presented a taxonomically complex problem. It has a wide distribution, variation patterns are complex and useful morphological characters are mainly quantitative. The taxonomic status of some of the entities recognised within it has been uncertain and subject to frequent change. *O. leptophyllus* was separated into five species and seven varieties in the "Flora of New Zealand Vol. I" (Allan 1961), and united into a single undivided but polymorphic species in the "Flora of New Zealand Vol. IV" (Webb 1988). Many botanists recognise different forms within this species based on their appearance and where they naturally occur, so the lack of available names in *Ozothamnus leptophyllus* for some species and varieties accepted under *Cassinia* in the "Flora of New Zealand Vol. I" (Allan 1961) can cause problems. There is a requirement for comprehensive revision, which will be undertaken in this study.

Ozothamnus leptophyllus has a wide geographical distribution, covering many ecological situations. As a common representative of New Zealand's flora it has been the subject of studies of vegetation (e.g., Betts 1920, Cockayne 1928, Wardle 1991), ecology (e.g., Betts 1920, Sheppard 1965, Lyon et al. 1971), and plant-insect relationships (e.g., Molloy 1959, Hoy 1962, Sheppard 1965, Primack 1983, Wilton 1997). Studies of the phenology (Sheppard 1965, Wilton 1997), growth (Wardle 1963, Sheppard 1965), cytology (Dawson & Beuzenberg 2000), anatomy (Betts 1920b, Breitwieser 1993, Wilton 1997), and flavonoid compounds (e.g., Breitwieser & Ward 1993, Reid & Bohm 1994, Wood et al. 1999) of *O. leptophyllus* have been undertaken, some of them with the aim of clarifying intrageneric and intergeneric relationships in the gnaphalioid Compositae. Although occasionally treated as a weed (e.g., Cunningham 1927a, Roy 1998), *O. leptophyllus* has even become a welcome member of New Zealand gardens.

The need for a detailed taxonomic investigation of *Ozothamnus* in New Zealand became quite obvious with a review of the relevant literature. Before undertaking a revision

it is worthwhile to compile and compare all available information about the object of research. This following introduction provides a detailed summary of all relevant literature concerned with *O. leptophyllus*. It tells the story about *O. leptophyllus* from its discovery more than 200 years ago to the present day.

1.1 Taxonomic history of *Ozothamnus leptophyllus* in New Zealand

The first collections of *Ozothamnus leptophyllus* (G.Forst.) Breitw. et J.M. Ward, as *Calea cinerea*, was made by Sir Joseph Banks (1743-1820) and Daniel Carl Solander (1733-1782) on Captain Cook's first voyage to New Zealand in 1768-71. Banks and Solander were enthusiastic collectors. Collecting was confined to a few places on the coast of the North Island from Poverty Bay to the Bay of Islands and to Queen Charlotte Sound and Admiralty Bay in the South Island. Altogether some 360 species of vascular plants were gathered, most coming from Tolaga Bay (160 spp.) and Queen Charlotte Sound (220 spp.). They collected among other plants a specimen Banks informally named *Calea cinerea*. A herbarium sheet, which is held with the Banks Herbarium at the Department of Natural History of the British Museum U.K., England, London (BM 000602390) (Appendix 1 Table 2), shows four different specimens of *Calea cinerea* collected by Banks and Solander. A note on the back of the herbarium sheet indicates where the specimens have been collected or where this plant has been recorded: at Teoneroa (Poverty Bay), on 8-11 October 1769, Tolaga (Tolaga Bay), on 23-29 October 1769, Opoorage (Mercury Bay), on 5-15 November 1769, Motu aro (Motu aro Island), on 29 November and 2 December 1769, Totara nui (Queen Charlotte Sound), on 15 January-6 February 1770). A coloured drawing (Catalogue Number NZ2/98 = New Zealand Volume 2 Folio 98 (Diment et al. 1987)) was prepared of a specimen of *Calea cinerea* from Poverty Bay by Sydney Parkinson, the botanical artist during Cook's voyage. In 1980, more than two hundred years after the historic voyage, Alecto Historical Editions in association with the Department of Natural History of the British Museum undertook the first complete printing of the collection of engravings of plants commissioned by Banks and based on Parkinson's drawings made during the voyage. Plate number 484 (Part XXIII New Zealand) from Banks' Florilegium (Banks et al. 1980-1990) shows *Calea cinerea* (as *Cassinia leptophylla* (G.Forst.) R.Br.)

(reproduced as the frontispiece of this thesis). The plants have for the most part been named and fully described by Solander at the time of collection but these names were never published. The original description of *Calea cinerea* can be found in Daniel Solander's "Plantae Australiae (Novae Zelandiae)" Volume I, pages 41-42 and 84-85 and in his Manuscript Slip Catalogue XVI page 303-305 (Diment et al. 1987). The New Zealand portion, which is entitled "Primitae Florae Novae Zealandiae", contains descriptions of nearly 360 species. Shortly before his death, Banks bequeathed his collections to Robert Brown who transferred them to the British Museum in 1823.

The New Zealand taxa now attributed to *Ozothamnus* were first mentioned in the published taxonomic literature in Forster's "Prodromus" (1786). Johannes Reinhold Forster and his son Georg Forster accompanied Cook on his second voyage to New Zealand, 1772-1775. Collections were made in Queen Charlotte Sound and Dusky Sound. In 1786 Georg Forster published his "Florulae Insularum Australium Prodromus", which contains diagnoses of 594 species, about 170 of which have New Zealand assigned as a locality. The descriptions are short and usually insufficient to allow identification of the species. Among these descriptions is the first published description of *Ozothamnus leptophyllus* as *Calea leptophylla*. G. Forster collected several specimens of *Ozothamnus leptophyllus*. Part of this collection is held at the herbarium of the Royal Botanic Gardens, Kew, London, U.K. A herbarium sheet contains three specimens, which were probably collected in Queen Charlotte Sound. Two other Forster specimens without any information about collecting date or locality are held in the Department of Natural History of the British Museum, London, U.K. (BM 000602391 and BM 00602392) (Appendix 1, Table 2). Unfortunately none of the specimens was in flower.

Calea leptophylla was transferred to *Cassinia* (as *C. leptophylla*) by Robert Brown (1817). However, *Calea leptophylla* was still mentioned in taxonomic literature by Achille Richard in 1832. He included in his publication descriptions of species that had been collected in the expeditions of D'Urville in 1824 and 1827 and most of those obtained by J.R. and G. Forster on Cook's second voyage. Among these is G. Forster's description of *Calea leptophylla*.

In 1826 a new form of *Cassinia* was discovered by Allan Cunningham. He spent four months making extensive and valuable collections from the Bay of Islands to Hokianga, and in the neighbourhood of Whangaroa, collecting 300 species, many of them

new, together with ample duplicates. In 1833 his brother Richard Cunningham, also a botanist, arrived in New Zealand. He also spent nearly five months travelling through the Bay of Islands, Whangaroa and Hokianga districts. A herbarium sheet (Allan Cunningham's New Zealand Herbarium No. 447) held at the herbarium of the Royal Botanic Gardens, Kew contains three specimens of *Cassinia* from different localities (Appendix 1 Table 2). One of these specimens, collected in 1826 by A. Cunningham, is labelled *Cassinia retorta*. This is the original collection for this species, described by A. Cunningham in 1837 (Cunningham in De Candolle 1837). An additional label on this specimen provides the information that it was collected at the Hokianga River. This label also contains the remark "distinct from *C. leptophylla* R.Br." and, in contradiction of the original label, gives R. Cunningham as collector and 1834 as collecting date. During a short visit to England in 1826, Allan Cunningham had prepared for publication an outline of the Flora of New Zealand, entitled *Florae Insularum Novae Zealandiae Precursor* but this was not published until much later (Cunningham 1839). In it he listed all of the species published by J.R. Forster (1786) including *C. leptophylla*. Cunningham (1839: 128-129) added to the description of *Cassinia leptophylla* the following observation: "Upon further examination of the specimens gathered on the shores of the Hokianga river in 1826, and at the period considered an unpublished species of *Cassinia*, I am now disposed to view it as Forster's plant". This was written before, but published two years after *Cassinia retorta* A.Cunn ex DC. had been already described and published by him as a new species (Cunningham in De Candolle 1837).

In the years 1840-1843 Edouard Fiacre Louis Raoul, a French surgeon to the two vessels "Aube" and "Allier", made collections, mainly at Akaroa but also in Queen Charlotte Sound. He was the first botanist to investigate the flora of the eastern side of the South Island and he described many new plant species from New Zealand. In 1844 Raoul's "*Choix de Plantes de la Nouvelle Zélande*" was published. It contains not only illustrations and descriptions of new plant species but also lists of the known species of the flora including, on page 45, *Cassinia leptophylla* and *Cassinia retorta*.

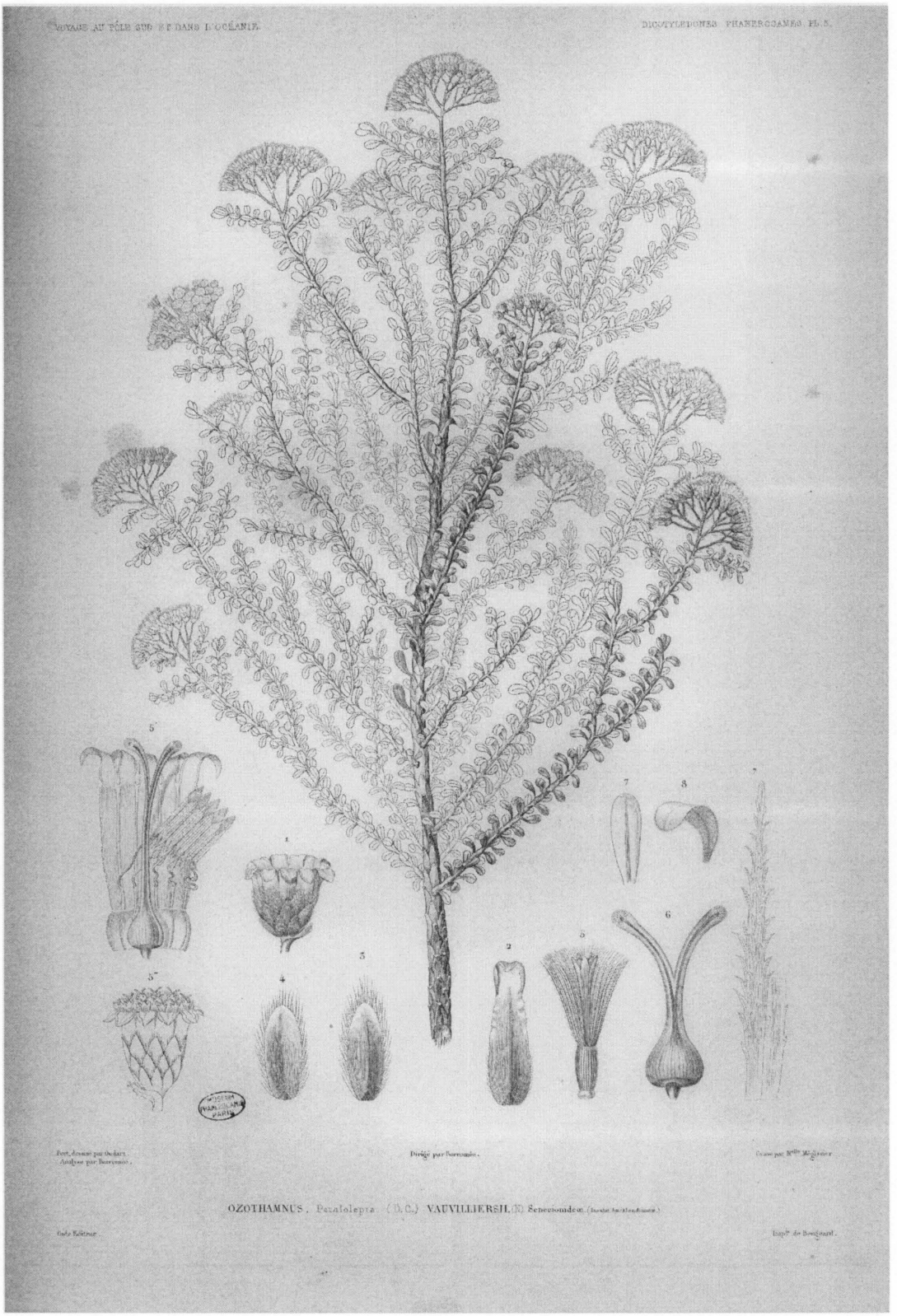


Fig. 1.1 *Ozothamnus vauvilliersii*, reproduced from Hombron &Jacquinot (1843-1853); "Voyage au Pole Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zélée, exécuté par ordre du roi pendant les années 1837-1838-1838-1840 sous le commandement de M.J. Dumont d'Urville, capitaine de vaisseau, Folio Atlas", Plate 5.

During the expedition of Admiral Jules Sébastien César Dumont D'Urville to the Antarctic region in the years 1839-1840, the two participating naturalists Jacques Bernard Hombron and Honore Jacquinot made the first collection of plants from the Auckland Islands. They collected a plant they called *Ozothamnus vauvilliersii*. The original collection is held in the Muséum National d'Histoire Naturelle France, Paris (P 00179961). The official record of the voyage (D'Urville 1841-1854) contains a folio atlas of botanical plates prepared under the direction of M. Hombron (Hombron & Jacquinot 1843-1853), and two volumes of descriptive matter. The second volume of descriptive matter (Hombron & Jacquinot 1853) contains a written description of *Ozothamnus vauvilliersii* Hombr. et Jacq. The Atlas was issued in 13 parts between 1843 and 1853 (Stafleu 1976: 698). Plate number 5 in the first issue (1843) shows *Ozothamnus vauvilliersii* (Fig. 1.1). The name *Ozothamnus vauvilliersii* was here validly published because the plate prepared by Hombron and Jacquinot is considered an "illustration with analysis" (sensu ICBN Ch. 4, Sec. 2, Art. 42.3 and Art. 44.1) and, therefore, the usual requirement for a written description or diagnosis (as specified in ICBN Ch. 4, Sec. 2, Art. 32.1) is waived.

Joseph Dalton Hooker was botanist to the Antarctic Expedition under Sir James Clark Ross on board the vessel "Erebus", which visited the Auckland Islands in 1840. Although the Auckland Islands had been visited by D'Urville during the previous year, nothing had been published about the vegetation and Hooker devoted himself to its exploration. He collected specimens that he labelled *Ozothamnus vauvilliersii* on the herbarium sheet (BM 000602394, BM 000602397). The first volume, "Flora Antarctica", of "The botany of the Antarctic voyage of H.M. discovery ships Erebus and Terror in the years 1839-1843, under the command of Captain Sir James Clark Ross" was published in 1847 and contains the first published written description of *Ozothamnus vauvilliersii* Hombr. et Jacq. (Hooker 1847) since Hombron and Jacquinot did not publish their written description of this plant until 1853 although the name was validly published in their Atlas in 1843.

In August 1841 the Ross expedition reached New Zealand and remained until November. During this time Hooker was actively engaged in collecting material for his projected "Flora of New Zealand", receiving assistance from William Colenso and Andrew Sinclair. Sinclair and David Lyall, who was attached as naturalist to the second vessel of the Ross expedition, collected *Cassinia leptophylla*. Hooker described in his second

volume of "The botany of the Antarctic voyage", the "Flora Novae-Zelandiae" (Hooker 1852), two *Cassinia leptophylla* varieties (*C. leptophylla* var. *β* and *C. leptophylla* var. *γ*) collected in Port Underwood and Canterbury respectively by Lyall. The "Flora Novae-Zelandiae" (Hooker 1852) contains, in addition to *C. leptophylla* and its varieties *β* and *γ*, *C. retorta* and *C. vauvilliersii* (Hombr. et Jacq.) Hook.f., the last transferred from *Ozothamnus*. *C. vauvilliersii* was recorded not only from the Auckland Islands but also from the North and South Islands, collected by John Bidwill and Lyall respectively. In the "Handbook of the New Zealand Flora" (Hooker 1864) *C. leptophylla* var. *γ* was raised to species level as *C. fulvida* Hook.f. Additional collections from various parts of New Zealand were noted. Hooker commented "I am still in some doubt as to the validity of this species, of which I had but one scrap when the Fl.N.Z. was prepared; the various specimens received since all agree with the original, and differ from *C. leptophylla* (to which, however, it is very nearly allied), by the strong fulvous colour and glutinous foliage" (Hooker 1864: 145).

John Buchanan, a resident of Dunedin, studied the native vegetation in the years 1860-1887, focussing on central and western Otago. He published 11 papers on new species in the "Transactions and Proceedings of the New Zealand Institute", including one in which he described a new species of *Cassinia*, *C. rubra* Buchanan, collected inland up the Wanganui River in the North Island (Buchanan 1887). He stated as a distinctive character the "bright pink or red involucral scales".

Another resident botanist and explorer, the Rev. William Colenso, also described several new plant species for New Zealand in the "Transactions and Proceedings of the New Zealand Institute". In a paper issued 1888 in Volume XX of the "Transactions" he described a plant he named *Olearia xanthophylla* collected in 1887 by H. Hill on the High Plains, Waimarino, on the west side of Mount Tongariro. This name is interpreted here and elsewhere (Kirk 1899; Cheeseman 1925, 1906; Allan 1961) as *Cassinia vauvilliersii*. Two years later, Colenso published *Cassinia spathulata* Colenso. He observed this plant in the years 1860-1889 on dry hills near the sea near Napier, Hawkes Bay and added the following note to his description: "I have long known this shrub, which grows naturally here in the borders of my paddock on the hill; but until this year I had always supposed it to be one of the described New Zealand species of *Cassinia* - probably *C. leptophylla*. This summer, however (in February), I was attracted to it by its charming and showy

appearance, so many hundreds of heads of pure-white flowers, formed by their large recurved scales; and on examination I found it to be a very distinct species. Its spathulate leaves yellowish below and sub-glutinous, larger heads of flowers, their coloured and woolly involucres, and their prominent white-tipped and largely-recurved floral scales, with the tops of the pappus flattened and coarsely serrate, form good differential characters" (Colenso 1890). Two herbarium sheets with the original collection of *Cassinia spathulata* are held in the herbarium of the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand: WELT 58873 (T. Kirk Herbarium No. 1237), WELT 24140.

Thomas Frederick Cheeseman was the next to discover and name a new *Cassinia* species, *C. amoena* Cheeseman. In 1896 he explored the flora of the North Cape District and found on cliffs near North Cape a plant which "differs from *C. leptophylla* in its much smaller size and different habitat, larger leaves, narrower heads with much fewer florets, and in the total absence of scales among the florets. It is much nearer to *C. vauvilliersii*, some forms of which approach it in habit. It can easily be distinguished, however, by the smaller size, narrower heads, fewer florets, and the absence of scales" (Cheeseman 1897). The original collection is held in the herbarium of the Auckland War Memorial Museum, New Zealand (AK 10298).

In 1899 Thomas Kirk's "Student's Flora of New Zealand and the Outlying Islands" was published. It recognises *C. retorta*, *C. leptophylla*, *C. rubra*, *C. vauvilliersii*, *C. amoena* and *C. fulvida*. Colenso's *Cassinia spathulata* was given only varietal rank as *Cassinia leptophylla* var. *spathulata* (Colenso) Kirk. Two additional *Cassinia* varieties made their appearance in Kirk's Flora: *Cassinia vauvilliersii* var. *albida* Kirk, distinguished from *C. fulvida* by the broader leaves and numerous scales among the florets, collected by Kirk at Mt. Fyffe (original collections: WELT 58218, WELT 29634 (T. Kirk Herbarium No. 1224), and AK 10304, AK 30904); *Cassinia fulvida* var. *linearis* Kirk, "distinguished from *C. retorta* and *C. leptophylla* by the fulvous or yellowish tomentum and the paucity or absence of scales amongst the florets", which was collected in 1896 by Bernard Cracroft Aston at the Southern Reservoir in Dunedin (original collections: WELT 58665, WELT 58747/A, WELT 58747/B, WELT 58748/A, WELT 58748/B, WELT 58749, and WELT 58755).

Cheeseman accepted in his "Manual of the New Zealand Flora" (1906) *C. retorta*, *C. leptophylla*, *C. rubra*, *C. vauvilliersii*, *C. amoena*, *C. fulvida*, and Kirk's *C. vauvilliersii*

var. *albida* and *C. fulvida* var. *linearis*. He provided the last of these with the remark "Very close to some forms of *C. leptophylla*, and only to be distinguished by the more fulvous viscid tomentum, fewer florets, and by the paucity or total absence of the scales among the florets". He reduced *C. rubra* to varietal rank as *C. vauvilliersii* var. *rubra* (Buchanan) Cheeseman and did not accept *C. spathulata*: "Mr. Colenso's *C. spathulata* does not seem to me to be even entitled to varietal rank" (Cheeseman 1906: 345).

Leonard Cockayne, who commenced his botanical explorations in 1887 and made many important contributions to our knowledge of the New Zealand flora, published in 1906 a paper on the subalpine scrub of Mount Fyffe (Seaward Kaikouras), in which he raised Kirk's *C. vauvilliersii* var. *albida*, which is confined to the Kaikoura Mountains and their vicinity, to species level as *C. albida*: "It is especially distinguished from *C. vauvilliersii*, of which Kirk considered it a variety, in the tomentum of the under surface of the leaf, which is white or yellowish-white, and not fulvous as in the latter species, and this character gives the shrub a most distinct appearance" (Cockayne 1906). He also described *Cassinia albida* var. *canescens* Cockayne: "There are two forms of *C. albida* - the one with a thin covering of hairs on the upper surface of the leaf, which is not noticeable without close examination, and does not in the least veil the green of the leaf; the other covered with a mat of fine hairs on the upper surface of the leaf, so as to give the whole plant the appearance of being covered with dust or afflicted with a mildew" (Cockayne 1906). He gives a detailed description of both plants including growth form and habit as well as ecological information. The original material of *Cassinia albida* var. *canescens* is held in the herbarium of the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand: WELT 58279 (D. Petrie Herbarium; ex L. Cockayne Herbarium No. 39), WELT 58358 (L. Cockayne Herbarium No. 9093).

One year after Cockayne's elevation of *C. vauvilliersii* var. *albida* to species rank, Cheeseman discussed its status, unconvinced that the species rank was justified. He remarked that all the New Zealand species of *Cassinia* are very closely allied and difficult to discriminate between (Cheeseman 1907). Cheeseman's "Illustrations of the New Zealand Flora" (1914) depicts on Plate 107 a drawing of *Cassinia amoena* (Fig. 1.2). In the description facing the drawing Cheeseman mentions "five or six well-ascertained species in New Zealand" and lists *C. retorta*, *C. leptophylla*, *C. fulvida*, *C. vauvilliersii*, *C. albida* and *C. amoena*.

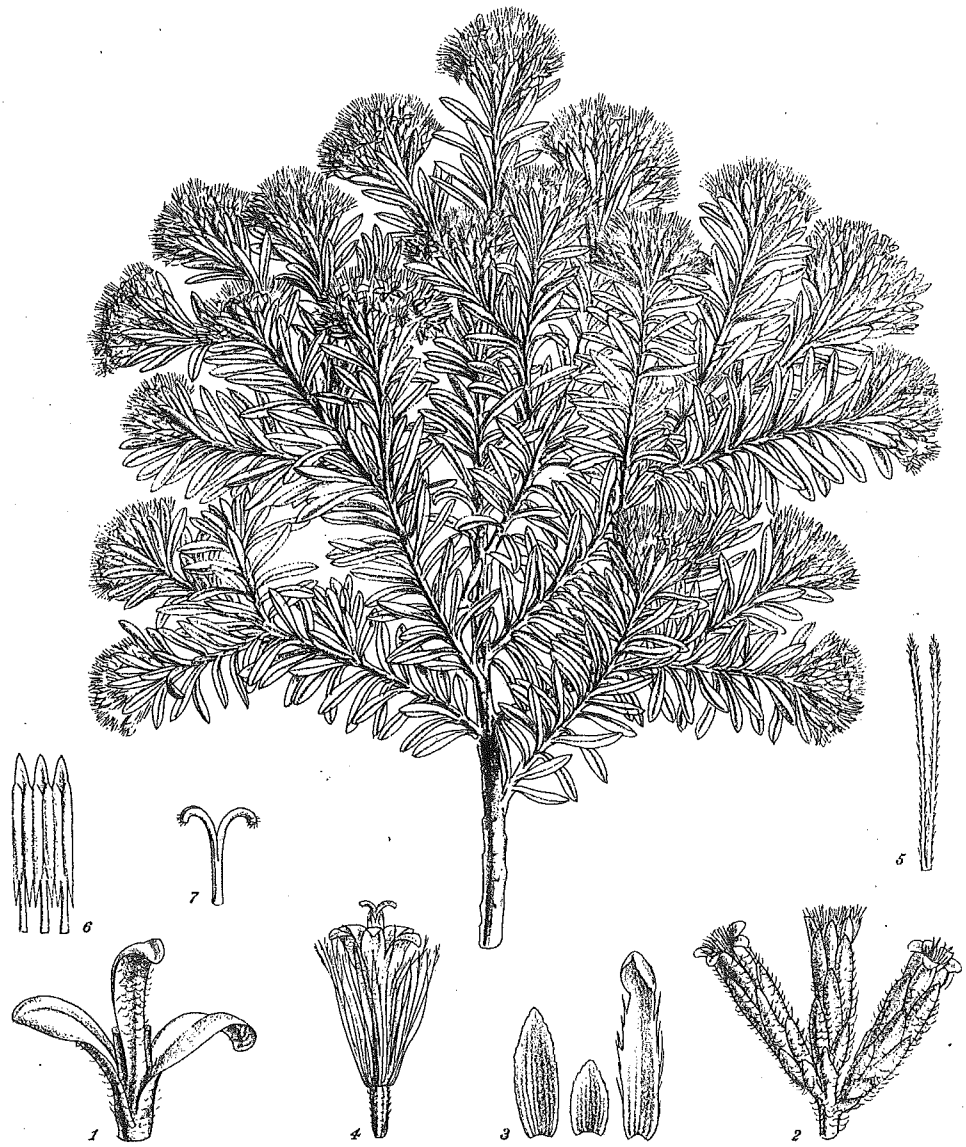


Fig. 1.2 *Cassinia amoena*, reproduced from Cheeseman (1914), "Illustrations of the New Zealand Flora", Plate 107.

In 1918 Cockayne wrote that he "would reverse" his decision to rank *C. albida* as a species, "were it not that both *C. vauvilliersii* and *C. albida* embrace more than one microspecies, and that if the latter were reinstated as a variety of the former it would be necessary to establish subvarieties in addition to varieties".

The treatment of *Cassinia* in the second edition of Cheeseman's "Manual of the New Zealand Flora" (1925) is identical to that in the first edition (Cheeseman 1906), except that Cheeseman remarked about *C. vauvilliersii* var. *albida*: "...at one time I was inclined to agree with Dr. Cockayne in definitely separating it from *C. vauvilliersii*. More recent study has led me to take the opposite view. And if I rightly understand Dr. Cockayne's remarks in Trans. N.Z. Inst. 1 (1918) 168, he also is inclined to reduce it to the position of a variety" (Cheeseman 1925: 988).

It was again Cockayne, this time together with Harry Howard Barton Allan, who discovered a new variety of *Cassinia*, *C. vauvilliersii* var. *serpentina* Cockayne et Allan, on the Mineral Belt in the Sounds-Nelson Botanical District (Cockayne & Allan 1926a). They added to their description of *C. vauvilliersii* var. *serpentina* the comment that the new variety of *C. vauvilliersii* is distinguished from *C. albida* (Kirk) Cockayne by the colour of the tomentum and the much smaller leaves. "It might quite well be thought an epharmonic form of *C. vauvilliersii*, due to the magnesian soil, but, if so, it is apparently "fixed", as it has kept unchanged to any extent for five years in cultivation" (Cockayne and Allan 1926b). The original collection of *C. vauvilliersii* var. *serpentina* is held in the herbarium of the Auckland War Memorial Museum, New Zealand (AK 31977).

In 1926 Harry Carse discovered and collected from a slope above Kerr Point in the North Cape District a hybrid between *C. amoena* and *C. retorta*: *Cassinia* x *amoenatorta* (Carse 1930). Carse distinguished three predominant forms of this hybrid: one very near to *C. amoena* but with shorter, more yellow leaves; a second form similar to the first one but with even smaller leaves; and a third form, which resembles *C. retorta* but with few florets in the capitulum.

C. fulvida var. *montana* was first mentioned by Cockayne in his "The Vegetation of New Zealand" as a plant of the *Festuca novae-zelandiae* association in the high mountain vegetation (Cockayne 1928: 305), but the name was not validly published since it was not accompanied by a diagnostic statement. Cockayne and Allan (1934) recognised *C. fulvida* var. *montana* when they listed *Cassinia* hybrids. Their list contains *Cassinia albida* x *fulvida* var. *montana* and *C. fulvida* var. *montana* x *vauvilliersii*, as well as Carse's *Cassinia* x *amoenatorta*, *C. albida* x vauvilliersii, and *C. retorta* x *vauvilliersii*.

Allan, in his "Flora of New Zealand Vol. I" (1961) accepted five species (*C. retorta*, *C. leptophylla*, *C. amoena*, *C. fulvida*, and *C. vauvilliersii*), and six segregate

varieties (*C. fulvida* var. *montana* Allan, *C. leptophylla* var. *spathulata*, *C. vauvilliersii* var. *albida*, *C. vauvilliersii* var. *canescens* (Cockayne) Allan, *C. vauvilliersii* var. *serpentina*, *C. vauvilliersii* var. *pallida* Allan) of which *C. vauvilliersii* var. *pallida* and *C. fulvida* var. *montana* were newly described. Allan collected the original material of *C. vauvilliersii* var. *pallida* at Robinson Creek, Upper Awatere Valley (Allan Herbarium, Landcare Research New Zealand, CHR 87404) in 1951. The original material of *C. fulvida* var. *montana* was collected by Victor D. Zotov in Arthur's Pass (Allan Herbarium, Landcare Research, New Zealand, CHR 9397) in 1936. Allan mentioned *C. vauvilliersii* var. *rubra* but added the remark that this is a plant with an "uncertain status". He did not accept Cockayne's *C. albida* as a species and *C. albida* var. *canescens* appears in his Flora as *C. vauvilliersii* var. *canescens*. *C. fulvida* var. *linearis* is not accepted and mentioned only under "Hybridism" in Allan's Flora: "(...) it may be of hybrid origin, but has not been critically studied" (Allan 1961: 729). Allan also mentioned Carse's *Cassinia* x *amoenatorta* and the hybrids previously listed in Cockayne and Allan (1934): "*C. retorta* also appears to cross with *C. vauvilliersii*, as does *C. leptophylla*. Within the *vauvilliersii* complex the vars. *pallida* and *albida* often meet and produce a polymorphic progeny. Field evidence suggests that the forms with fulvous tomentum may hybridise with var. *pallida*; more certain is the crossing of *C. fulvida* var. *montana* with *C. vauvilliersii*, the reputed hybrids showing varying degrees of glutinosity and of the number of white-tipped receptacular scales" (Allan 1961: 729).

Colin J. Webb in the "Flora of New Zealand Vol. IV" (1988) recognised only a single polymorphic New Zealand species, *Cassinia leptophylla*. He agreed that *C. leptophylla* displays considerable local differentiation but remarked that there are no characters that clearly delimit more than one species. Characters such as leaf shape and size, and number of receptacle scales have been found to vary continuously within and between *Ozothamnus leptophyllus* populations, so that many plants are not clearly referable to any previously accepted species (Webb 1988). He remarked that "Distinctive local populations may be defined by combinations of characters, (...), but these do not merit recognition at sp. rank. The division of *C. leptophylla* into subspp. or vars. is probably warranted, but a satisfactory treatment must await a more detailed analysis of this variable sp." (Webb 1988: 233).

Breitwieser and Ward (1997) demonstrated that the closest relatives of the New Zealand *C. leptophylla* are within *Ozothamnus* and made the taxonomic change to *O. leptophyllus*.

1.2 Generic position of the *Ozothamnus leptophyllus* complex in New Zealand

The generic position of the *Ozothamnus leptophyllus* complex in New Zealand was discussed by Breitwieser and Ward (1997). The following account is taken largely from that paper.

Both *Cassinia* and *Ozothamnus* were erected in 1817 by Robert Brown to contain two morphologically similar groups of shrubby Australasian Compositae having leaves with recurved margins, corymbose or paniculate terminal inflorescences, capitula with papery white, ash-grey, or gold involucral bracts, a small number of florets either all tubular and hermaphrodite or with few narrow female ones at the circumference, anthers with tails, style arms with blunt bristly apices, and persistent pappus. *Ozothamnus* was distinguished from *Cassinia* by the absence of paleae on the receptacle among the florets.

Brown (1817) distinguished two sections of *Cassinia*, the first characterised by a spreading involucre and containing only *Cassinia leptophylla* from New Zealand, the second characterised by a converging involucre and containing all the shrubby Australian species of *Cassinia*. Bentham (1873) assigned the New Zealand specimens of *Cassinia* to the subgenus *Rhynea*, together with one species from South Africa, now assigned to the genus *Tenrhynea* Hilliard & Burtt (Hilliard & Burtt 1981).

Hooker (1852, 1860, and 1864) identified several characters shared between *Ozothamnus* and New Zealand *Cassinia*. He appended to the description of *Cassinia vauvilliersii* in his "Florae Novae-Zelandiae" (1852) the following comments: "It so very closely resembles the *C. cuneifolia* A.Cunn. (in DC. Prodr.), of Mount Wellington, in Tasmania, that were that plant a true *Cassinia* I should possibly have united them, but the latter having no paleae on the receptacle amongst the flowers, it must be referred to *Ozothamnus*" (Hooker 1852: 133). In his "Flora Tasmaniae" (1860) he remarked of

Ozothamnus backhousei: "A very well marked species, and like no other Australian one, but so similar to the New Zealand *Cassinia Vauvilliersii*, that the presence of paleae on the receptacle of the plant is almost the only character whereby it can be distinguished" (Hooker 1860: 204). And in his "Handbook of the New Zealand Flora" (1864) he commented on *C. fulvida*: "I find few or no scales amongst the florets, so, by right, the plant should perhaps be referred to *Ozothamnus*" (Hooker 1864: 145).

Hooker (1852, 1855) included the shrubby New Zealand species *Ozothamnus coralloides, O. depressus, O. glomeratus, O. microphyllus,* and *O. selago* in the genus *Ozothamnus*. Later Bentham (1866, 1873) relegated this genus to a section of *Helichrysum*, but Anderberg (1991) once more recognised it at generic level. However, the New Zealand shrubby species of *Helichrysum*, treated in *Ozothamnus* by Anderberg (1991) are not congeneric with the Australian species of *Ozothamnus* (Breitwieser & Ward 1993, Breitwieser & Ward 1997) and are closer to some other New Zealand taxa than they are to *Ozothamnus*.

Breitwieser and Ward's (1997) transfer of *Cassinia leptophylla* to *Ozothamnus* was the result of intensive research on the systematics of the New Zealand gnaphalioid Compositae, which demonstrated that *Cassinia leptophylla* was congeneric with the Australian *Ozothamnus*. The leaf anatomy of *Cassinia fulvida* and *C. leptophylla* sensu stricto of New Zealand was found to be almost identical to that of *Ozothamnus rodwayi* of Tasmania (Breitwieser 1993). Pollen morphology also linked *C. leptophylla* to *Ozothamnus* rather than *Cassinia* (Breitwieser and Sampson 1997a,b). Characters which are shared by New Zealand and Tasmanian representatives of *Ozothamnus* and distinguish them from Tasmanian species of *Cassinia* are: leaves not grooved above the midvein and lacking biserate trichomes with very broad bases; capitula in smaller, more rounded clusters; individual capitula longer, and constricted slightly below the apex; involucral bracts radiating; achenes longer; apical cells of the pappus hairs broader (Breitwieser & Ward 1997).

Both *Ozothamnus* and *Cassinia* belong to the gnaphalioid group of Compositae, and were placed in the subtribe Gnaphaliinae (tribe Inuleae) by Bentham (1873). The systematics of New Zealand gnaphalioid Compositae was discussed by Ward and Breitwieser (1998). The following account is based largely on this paper.

Major changes in classification within this group of Compositae have been proposed since the publication of the most recent New Zealand Floras treating dicotyledons (Allan1961, Webb et al. 1988). Seven indigenous genera were accepted by Allan (1961) in the tribe Inuleae Cass. Six of them, *Cassinia* R.Br., *Ewartia* Beauverd, *Gnaphalium* L., *Helichrysum* Mill., *Leucogenes* Beauverd, and *Raoulia* Hook.f., were referable, following Bentham (1873), to the subtribe Gnaphaliinae and one, *Craspedia* G.Forst., to the subtribe Angianthinae. In the fourth volume of the "Flora of New Zealand" (Webb et al. 1988) ten genera were accepted, with *Anaphalis* DC, *Haastia* Hook.f., and *Pseudognaphalium* Kirp. being additional to those accepted by Allan (1961). Of the species recognised in *Gnaphalium* by Allan, *Pseudognaphalium luteoalbum* was accepted, following Hilliard & Burtt (1981), and the "anaphalioid" species of *Gnaphalium* (Drury 1970) were transferred to *Anaphalis* by Webb (1987). Merxmüller et al. (1977) transferred *Haastia* from another tribe (Astereae).

Anderberg (1989) regards the tribe Inuleae sensu lato as paraphyletic. He divided it into three tribes, with all New Zealand genera falling into the largest of these, the Gnaphalieae. Genera recognised in New Zealand by Anderberg (1991) were *Cassinia*, *Raoulia*, *Leucogenes*, *Pseudognaphalium*, *Craspedia* (all recognised by Webb 1988), *Anaphalioides* (Benth.) Kirp. (following Kirpicznikov (Kirpicznikov & Kuprijanova 1950) and not Webb (1987) who assigned the "anaphalioid" species of *Gnaphalium* to *Anaphalis*), *Euchiton* Cass. (comprising some species formerly assigned to *Gnaphalium*, following Cassini (1828)), *Ozothamnus* R.Br. (comprising the woody species of *Helichrysum*), *Psychrophyton* Beauverd (comprising some species formerly assigned to *Raoulia*), and *Ewartiothamnus* Anderb., a monotypic genus to which *Ewartia sinclarii* was transferred. *Haastia* was not mentioned by Anderberg (1991). The herbaceous New Zealand species formerly in *Helichrysum* (*H. bellidioides* (G.Forst.) Willd. and *H. filicaule* Hook.f.) were assigned to the "Scorpioides complex" of the Australian genus *Lawrencella* Lindl., but no formal combinations were made.

Anderberg (1991) distributed the New Zealand taxa over four of the five subtribes of the Gnaphalieae recognised by him. *Anaphalioides*, *Cassinia*, *Ewartiothamnus*, *Ozothamnus* and *Raoulia* were assigned to the Cassiniinae Anderb.; *Euchiton*, *Leucogenes* and *Pseudognaphalium* to the Gnaphaliinae; *Psychrophyton* to the Loricariinae Anderb.; and *Craspedia* and the herbaceous species formerly in *Helichrysum* to the Angianthinae

Benth. (Anderberg 1991). Anderberg's subtribes separate closely related (Ward 1993b, Breitwieser & Ward 1993, Glenny & Wagstaff 1997) New Zealand genera at the subtribal level. The subtribes proposed for the Gnaphalieae by Anderberg (1991) are not accepted by New Zealand taxonomists working on the classification of this group, and nor are the genera *Ewartiothamnus, Psychrophyton* and *Ozothamnus* sensu Anderberg, pending revision of generic limits in New Zealand Gnaphalieae (see Ward & Breitwieser 1998).

Since the publication of Anderberg's (1991) revision of the Gnaphalieae, several contributions to increasing the understanding of the relationships in the New Zealand Gnaphalieae have been provided.

Developments involving names of New Zealand genera are the transfer of the indigenous species of *Cassinia* to *Ozothamnus* (Breitwieser & Ward 1997), the describing of a new, monotypic genus, *Rachelia* J.M.Ward & Breitw. (Ward et al. 1997), and the revision of what were then *Helichrysum bellidioides* and the indigenous species of *Anaphalis*, all of which are now referred to *Anaphalioides* (Glenny 1997).

Other important studies on the systematics of New Zealand gnaphalioid Compositae focus on morphology and phenetics (Ward 1993a, 1993b), on leaf anatomy, flavonoid profiles and pollen (Breitwieser 1993, Breitwieser & Ward 1993, Breitwieser & Sampson 1997a, 1997b), on cytology (Dawson et al. 1993), on phytochemistry (Reid & Bohm 1994, 1995) and on phylogenetic analyses of DNA sequence data (Glenny & Wagstaff 1997, Breitwieser et al. 1999). Most of these studies include *Ozothamnus leptophyllus*.

In her numerical phenetic study of *Raoulia* in relation to allied genera Ward (1993b) included from what is now *Ozothamnus leptophyllus* two forms treated by Allan (1961) as *C. fulvida* and *C. leptophylla* (sensu stricto). The two forms clustered together in the average linkage phenogram and also formed a ball cluster in the numerical phenetic analysis of 97 individuals representing all species of *Raoulia*, *Ewartia*, and *Leucogenes*, and selected species of *Anaphalioides* (as *Anaphalis*), *Ozothamnus* (as *Cassinia*), *Euchiton* (as *Gnaphalium*), *Helichrysum*, *Mniodes*, and *Pseudognaphalium*. *Ozothamnus* appeared to be distinct from all other New Zealand genera (Ward 1993b). Breitwieser (1993) described the leaf anatomy of 47 taxa of gnaphalioid Compositae from New Zealand and Tasmania. She included in her studies the same two forms of *Ozothamnus leptophyllus* (as *Cassinia fulvida* and *C. leptophylla*). In most of the 52 examined anatomical characters the two New

Zealand forms of *Ozothamnus* are identical. However, some differences could be observed in leaf width, thickness of palisade chlorenchyma, thickness of spongy chlorenchyma, length of palisade cells of the first row, and the number of palisade layers. Breitwieser & Ward (1993) subjected 45 species of the Gnaphalieae (as Inuleae), predominantly from New Zealand and Tasmania, to numerical phenetic analysis using 49 leaf anatomy and 38 leaf flavonoid characters. The leaf flavonoid data made it clear that *Ozothamnus leptophyllus* is closer to the Australian species of *Ozothamnus* (*Ozothamnus rodwayi* as *Helichrysum backhousei* and *O. obcordatus* DC. as *Helichrysum obcordatum* (DC.) F.Muell.) than it is to the Australian species of *Cassinia*. The distribution of the leaf flavonoids of the two forms of *Ozothamnus leptophyllus* (as *Cassinia fulvida* and *C. leptophylla*) was identical. Only the relative concentration (as judged visually) seemed to vary for some compounds between the two forms (Breitwieser & Ward 1993). The numerical analysis based on leaf anatomy helped to visualise the results already presented by Breitwieser (1993). The combined phenogram based on leaf flavonoid and leaf anatomy showed that the New Zealand forms of *Ozothamnus leptophyllus* (as species of *Cassinia*) were again allied with the Australian species of *Ozothamnus* (as *Helichrysum* sect. *Ozothamnus*) and more distantly with the Australian species of *Cassinia* (Breitwieser & Ward 1993). In pollen studies Breitwieser and Sampson (1997a,b) discovered further characters that link New Zealand forms of *Ozothamnus* (as species of *Cassinia*) with Tasmanian *Ozothamnus* rather than with Tasmanian or mainland Australian species of *Cassinia*. Breitwieser and Sampson (1997a,b) also included in their pollen studies the two forms of *Ozothamnus* from New Zealand (as *Cassinia fulvida* and *C. leptophylla*), and discovered differences in the pollen morphology between these two forms: *C. fulvida* differs from *C. leptophylla* by having shorter spines with a shallow angle and obtuse spine tips (Breitwieser & Sampson 1997a).

Wilton (1997) described the stem anatomy of 51 Gnaphalieae (as Inuleae) from New Zealand and Tasmania and used anatomical features as evidence for systematic relationships. One of the species he included in his study was *Ozothamnus leptophyllus*. Numerical and cladistic analysis based on stem anatomy showed the close association of *O. leptophyllus* to the Tasmanian species of *Ozothamnus* and supported the transfer of this species from *Cassinia* (Breitwieser & Ward 1997). The analysis of the stem anatomy indicated further that the differences between *Ozothamnus* and *Cassinia* are slight and that

there is not a close affinity between *Ozothamnus* and the woody New Zealand *Helichrysum* species.

Ozothamnus leptophyllus was included in the DNA sequencing study by Glenny and Wagstaff (1997). The objective of their study was to determine whether New Zealand *Anaphalioides* (as *Anaphalis*) are monophyletic and whether other New Zealand Gnaphalieae or the predominantly Northern Hemisphere genus *Anaphalis* are the group's closest relatives. They also aimed to gain insights into the biogeography of the New Zealand members of the tribe Gnaphalieae, in particular with respect to the number of occasions ancestors of New Zealand members have arrived in New Zealand and whether an ancestor of New Zealand *Anaphalioides* (as *Anaphalis*) has arrived separately from other New Zealand Gnaphalieae. The maximum likelihood tree in their study showed *O. leptophyllus* in a group together with *Craspedia* species. Both of them are New Zealand species but in genera that are present and probably originated in Australia (Glenny & Wagstaff 1997). Another more extensive study to test hypotheses of relationships in New Zealand Gnaphalieae using DNA evidence (Breitwieser et al. 1999) showed *Ozothamnus leptophyllus* 1) as a separate member of the Australasian polytomy in a strict consensus tree of a parsimony analysis and 2) as the sister taxon of the *Pycnosorus/Craspedia* clade in a majority rule consensus tree. It was isolated in the maximum likelihood analysis. Breitwieser et al. (1999) came to the conclusion that *Ozothamnus leptophyllus* is clearly not closely related to any of the taxa studied from the genera *Craspedia*, *Pycnosorus*, *Euchiton*, *Ewartia*, *Pterygopappus*, *Rachelia*, *Leucogenes*, *Raoulia*, *Helichrysum*, *Anaphalioides*, *Anaphalis*, and *Pseudognaphalium*, but belongs within the mainly Australian genus *Ozothamnus*.

Research on the New Zealand Gnaphalieae continues with several groups presently under revision, including *Craspedia* (I. Breitwieser), *Raoulia* (J. Ward), the whipcord species of *Helichrysum* (J. Ward), and the *Ozothamnus leptophyllus* complex (this study).

1.3 Distribution and ecology of *Ozothamnus leptophyllus*

Ozothamnus leptophyllus occurs throughout New Zealand. The forms previously described as five species in *Cassinia* vary in their distribution patterns (Fig. 1.3).

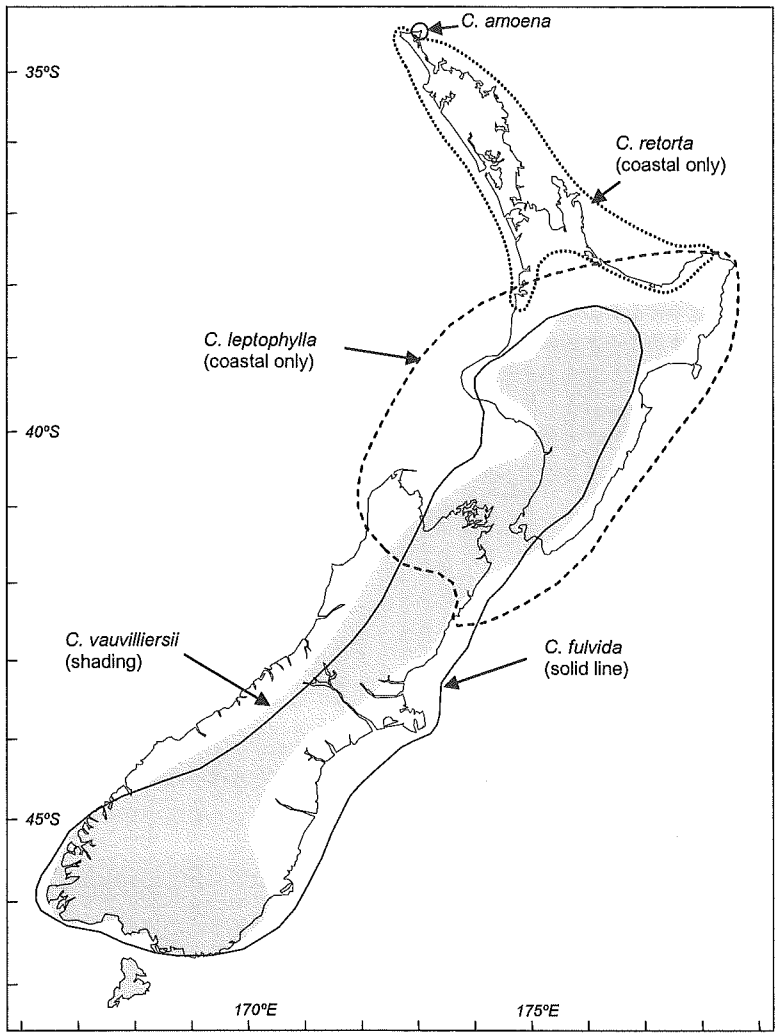


Fig. 1.3 Approximate distribution of '*Cassinia*' species on the three main islands of New Zealand. Modified from Sheppard (1965).

The following distributions are given in literature for '*Cassinia*' species.
'*C. amoena*' is the northernmost species, known only from the North Cape area between Surville Cliffs and Kerr Point (Cheeseman 1897, Kirk 1899, Cheeseman 1906, Cheeseman 1914, Cheeseman 1925, Allan 1961). '*C. retorta*' covers the Auckland provincial area as far south as latitude 38°30'S (Hooker 1852, Hooker 1864, Kirk 1899, Cheeseman 1906, Cockayne 1910, Cheeseman 1925, Allan 1961). At approximately latitude 37°30'S,

'*C. retorta*' overlaps in its distribution with '*C. leptophylla*'. '*C. leptophylla*' occupies a central portion of New Zealand, occurring in Taranaki, Hawkes Bay, the Cook Strait area, northern Marlborough, and in parts in Nelson. It extends southwards coastally to latitude 42°S. '*C. fulvida*' has a wide geographic distribution. From latitude 38°S it is common in subalpine scrub and grassland, and extends its range to include littoral habitats in Marlborough and Canterbury north of the Banks Peninsula (Hooker 1852, Hooker 1864, Kirk 1899, Cheeseman 1906, Cheeseman 1914, Cheeseman 1925, Allan 1961). '*C. vauvilliersii*' has the widest latitudinal (and altitudinal (see below)) range of any of the '*Cassinia*' species. It extends from 37°S to the Auckland Islands (Hombron & Jacquinot 1853, Hooker 1847, Hooker 1852, Hooker 1864, Colenso 1888, Kirk 1899, Cheeseman 1906, Cheeseman 1914, Cheeseman 1925, Allan 1961).

Ozothamnus leptophyllus covers many ecological situations, which are not only mentioned in the Floras and taxonomic literature but also in literature dealing with the vegetation of New Zealand such as Cockayne (1910 and 1928), Martin (1932) and Wardle (1991). Cockayne (1910 and 1928) and Wardle (1991) describe a whole range of different vegetation types in which *O. leptophyllus* is present. Both authors distinguish between different '*Cassinia*' species (Cockayne 1928) or at least different "forms" based on where they occurred. Wardle (1991), recognised one polymorphic species of '*Cassinia*' in New Zealand after Webb (1988), but also distinguished between different "forms".

'*Cassinia retorta*', '*C. amoena*', and '*C. leptophylla*' are ecologically restricted to littoral habitats. '*C. fulvida*' extends inland and ascends to the montane belt, but is also coastal in the South Island. '*C. vauvilliersii*' is common in subalpine shrubland and grassland, but descends to low altitudes in the southernmost parts of the South Island, Stewart Island, and the Auckland Islands.

Within all the above localities and habitats, *Ozothamnus leptophyllus* is present only in areas of open scrub or thicket, never in dense closed scrub. It is often found at the edges of erosion gutters or in avalanche chutes. *O. leptophyllus* is recognised as having a high light intensity requirement. It is weakened and eventually suppressed by strongly growing tussock grasses, but thrives where the ground cover has been removed. *O. leptophyllus* was found to be suppressed by reduced light and nutrients reaching the plant due to competition by surrounding tussocks (Sheppard 1965).

1.3.1 Natural plant communities with *Ozothamnus leptophyllus*

In plant communities of the coastal vegetation (dunelands, rock and cliff vegetation), *Ozothamnus leptophyllus* is one of the early shrub species in primary succession (Sheppard 1965, Wardle 1991). The ecologically-equivalent "species" of '*Cassinia*' ('*retorta*', '*leptophylla*', '*fulvida*') are respectively the dominant or sole form in shrub dunes, fixed dunes, hollows and sand-plains of the Northern, Central and Southern botanical provinces (Cockayne 1928). Other "primeval" (without permanent human habitation or modification) habitats in which '*C. fulvida*' and '*C. vauvilliersii*' occur as an early shrub species of a primary succession are edges of riverbeds, slips, and avalanche tracks (Sheppard 1965). *Ozothamnus* scrub present in high mountain habitats appear also to be of unmodified nature (Cockayne 1928). Cockayne (1928) lists and describes several, mainly subalpine communities in which one or the other '*Cassinia*' species occurs:

1) *Hebe* scrub, an association usually of shingly ground distinguished by the dominance of one or more species of *Hebe*;

2) Shrub-composite scrub, distinguished by the dominance, or occasionally subdominance only, of shrubby or stunted arboreal species of one or both of *Olearia* and *Senecio*, various divaricating shrubs, fastigiate species of *Dracophyllum*, *Phormium colensoi*, '*Cassinia vauvilliersii*', *Phyllocladus alpinus* and *Hebe*. Shrub-composite scrub requires a high rainfall for its full development. In North Island, it occurs on Mt. Hikurangi, the Ruahine and Tararua Ranges and Mt. Egmont. In South Island, it is a characteristic feature of the Western district on both sides of the Divide, making, in many places, a broad belt above the forest and partly filling the cirques at the sources of glacial rivers;

3) *Dracophyllum* scrub, which occurs only on dry South Island mountains, consisting of *Dracophyllum uniflorum*, *Hebe traversii,* species of *Cassinia*, *Olearia cymbifolia*, *Podocarpus nivalis* and *Helichrysum microphyllum*;

4) *Cassinia* scrub, with '*Cassinia vauvilliersii*' dominant, accompanied by *Dracophyllum uniflorum*, *Aciphylla maxima* and *Gaultheria rupestris*, and in open spaces amongst rocks, various characteristic high-mountain shrubs, including *Olearia moschata* and *Senecio cassinioides*.

Beside these four scrub communities, mixed communities (shrubs, herbs, semi-woody plants, grasses etc.) and herb-moor communities, can contain '*Cassinia*

vauvilliersii', '*C. fulvida* var. *montana*' or '*C. albida*' (Cockayne 1928). Wardle (1991) mentioned '*C. vauvilliersii*' also as a shrub of mountain wetlands and cushion bogs on the southern borders of the Otago plateaus.

Cockayne (1928) was sceptical about the virgin status of the high mountain (montane and subalpine) communities. He remarked: "However, dominance of *Cassinia* generally means there has been burning and the association it governs must always be looked upon with suspicion" (Cockayne 1928: 281). Burning of tall subalpine woody vegetation often led to prolonged replacement by low shrubs, tussock grasses and large herbs. Wardle (1991) mentioned the subalpine secondary heath as a form of high-altitude secondary succession. Subalpine heath regenerating after fire is scattered through mountainous regions, but is most extensive east of the Main Divide in the South Island. Subalpine grasslands have extensively replaced beech forests, mixed forests and primary heaths burnt over the last 1000 years. In the resulting secondary shrubheath '*Cassinia*' is more abundant than in primary heath, and may dominate in gullies and during early stages of succession (Wardle 1991).

1.3.2 *Ozothamnus leptophyllus* as an "indigenous-induced" member of secondary succession

The vast majority of plant communities with *Ozothamnus leptophyllus* represent stages of secondary succession, or, according to Cockayne (1928), are "indigenous-induced". *Ozothamnus* invades grassland beyond the range of *Leptospermum scoparium* and *Kunzea ericoides*, being more tolerant of coastal exposures, high altitudes and competition from grasses, but probably less drought tolerant (Wardle 1991). *Ozothamnus* bushes are short-lived and in the absence of fire are succeeded by longer-living plants. *Leptospermum scoparium* and *Kunzea ericoides* eventually outgrow and suppress *Ozothamnus* plants that establish at the same time (Sheppard 1965).

The shrubland communities of the lowlands and lower hills (lowland-montane belt) occur under diverse conditions (Cockayne 1928). They are equally present in areas of high and low precipitation. Soil may be alluvial of various kinds, clays, loess, sand, gravel,

calcareous or non-calcareous and pumice of different sorts. Though frequently dominant on poor soils this is more a consequence of absence of competitors than preference for less fertile soils. The water content of the substrate differs greatly according to rainfall and its water holding capacity, and fluctuates considerably both at different seasons and even over brief periods (Cockayne 1928). In certain localities, bog conditions are present and it is not easy to differentiate between shrubland proper and bog-shrubland (Cockayne 1928).

Both Cockayne (1928) and Wardle (1991) described several "indigenous-induced" plant communities with '*Cassinia*'. Most of them are *Leptospermum-Pteridium* (manuka-bracken) communities, like the two ericoid shrubland types, *Leptospermum* (manuka) shrubland, and the *Cassinia* shrubland.

The *Leptospermum* (manuka) shrubland

In the *Leptospermum* shrubland the species differ considerably according to botanical district and decrease in number from north to south. Cockayne (1928) distinguished between the *Leptospermum* shrubland of the southern North Island and the South Island. The chief distinction of the South Island *Leptospermum* communities is the presence, frequently in abundance, of *Discaria toumatou*, '*Cassinia fulvida*', '*C. vauvilliersii*', '*Cassinia*' hybrids, and species of *Carmichaelia*.

Ozothamnus in *Leptospermum* shrubland on ultramafic surfaces

A special type of *Leptospermum* shrubland has been distinguished by Betts (1920), Cockayne (1928), and Wardle (1991), on ultramafic surfaces such as the Magnesian soil shrubland of the Mineral belt of the Sounds-Nelson Botanical District and the Surville Cliffs. A local endemic '*Cassinia*' has been described from both of these localities. '*Cassinia amoena*' is only found on the Surville Cliffs where clay soils derived from ultramafic rocks support low, fire-modified scrub dominated mainly by *Leptospermum scoparium*, *Hebe ligustrifolia* and '*Cassinia amoena*' (Cheeseman 1897, Kirk 1899, Cheeseman 1906, Cheeseman 1914, Cheeseman 1925, Allan 1961). '*Cassinia vauvilliersii* var. *serpentina*' is recorded from Dun Mountain, near Nelson, the widest part of the Mineral Belt (Cockayne & Allan 1926). The belt is a narrow, frequently stony tract, consisting of peridotite and serpentine rocks, extending for about 96 km from D'Urville Island to the western part of the Nelson-Sounds district. The vegetation of the belt presents

a contrast to that of the adjacent forests and botanical accounts of this area include those of Betts (1918, 1919, 1920). The autecology of certain plants was studied by Betts (1918, 1919, 1920). She described the habit and anatomy of a '*Cassinia*' from the Mineral Belt, which she called *Cassinia vauvilliersii* var. *rubra* (Betts 1920). The '*Cassinia*' specimens found in the area occupied by the Mineral Belt are, according to Betts (1920), not modified in form compared to the usual type.

Lyon et al. (1968, 1970, 1971) carried out research on the tolerance of some New Zealand plants to ultramafic and serpentine soils of the Dun Mountain region. Six species and soil samples were analysed for calcium, magnesium and the trace elements cobalt, chromium, copper and nickel. One of the chosen species was the serpentine ecotype of '*Cassinia vauvilliersii*', '*Cassinia vauvilliersii* var. *serpentina*'. Most of the species included in this study differed in their ability to accumulate or exclude soil elements. '*Cassinia vauvilliersii* var. *serpentina*' was intermediate in many respects (Lyon et al. 1971) but showed highly significant correlation between plant ash and soil concentration for chromium, cobalt and nickel. Therefore a genotypic variation between forms of '*Cassinia*' from sites of different mineral content seemed unlikely (Lyon et al. 1968).

'*Cassinia*' shrubland

The other ericoid indigenous-induced shrubland community of the lowland-montane belt is called '*Cassinia* shrubland' Cockayne 1928, Wardle 1991). *Ozothamnus leptophyllus* is the dominant plant in this community, which occurs frequently, both near the coast and inland as far as the montane or even the lower subalpine belt. In parts of North Island and the Sounds-Nelson district, it occupies wide areas where forest has been felled and burned or burned standing (Cockayne 1928).

1.3.3 *Ozothamnus leptophyllus* and agriculture

As early as 1874, only 100 years after the first discovery of *Ozothamnus leptophyllus* in New Zealand, Travers wrote an article "On the spread of *Cassinia leptophylla*" (Travers 1874). He observed the increase of '*C. leptophylla*' on the hills on the eastern side of Wellington Harbour, the Miramar Peninsula and in Nelson and explained the expansion of

'*C. leptophylla*' with increased production of fertile seeds, due in part to the spread of European bee pollinators, the disturbance of the soil by treading of animals, and the "destruction or diminution in number of some form of insect life which formerly fed upon its flower heads" (Travers 1874).

Ozothamnus leptophyllus is considered a serious weed of hill country grassland due to its small, pappus-bearing fruits that are widely dispersed in the wind, their high viability, an ability for seedlings to establish within grass swards, the rapid growth of the seedlings and an ability to resprout from the base after fire (Sheppard 1965). It is not palatable to stock (Sheppard 1965) and control methods appear problematic due to its high tolerance to herbicides (Roy 1998) and its ability to throw up fresh shoots from the base of the stem if the shrub has been burnt or slashed and to colonise burnt areas rapidly by the wind-borne seeds.

A positive aspect of *O. leptophyllus* in agriculture is the value of the plants in providing cover on bare or denuded ground, thus lessening chances of erosion. It could be used to aid the transformation of denuded land to agricultural use and to stabilise erosion slopes (Sheppard 1965).

1.4 Other information on *Ozothamnus leptophyllus* in New Zealand

1.4.1 Parasites

Numerous parasitic plants, fungi and insects have been recorded from *Ozothamnus* in New Zealand but none of them seems to be able to affect the general fitness of populations. It is likely that plant exudates act to discourage the attentions of potential pests and parasites (Kelsey et al. 1984).

1.4.1.1 Parasitic plants and fungi

Cockayne (1928) reported that '*Cassinia amoena*' is one of the species parasitised by *Cassytha paniculata* R.Br. (Lauraceae), a twining leafless plant, the most common hosts of which occur among shrubby species of the Auckland 'gumlands' (Cockayne 1928).

A fungus attacking *O. leptophyllus* was observed by Cunningham (1927a). He noted that wind-damaged shrubs on a property at Weraroa, Wellington, were attacked by a shelf fungus (Polyporaceae), which appeared to be killing the plants. The fungus was first identified as *Fomes ignarius* and later changed to *Fomes robustus* P. Karst (Cunningham 1927b). At the time *Fomes robustus*, today known as *Phellinus robustus* (P.Karst.) Bourdot & Galzin, had been considered as a means for controlling *Ozothamnus* (Cunningham 1927a). Two other records of *Phellinus robustus* with '*C. leptophyllus*' as host plant were found: Dingley (1969) and Pennycook (1989). Pennycook also lists another fungus, *Capnodium walteri* Sacc., which was recorded by Hughes (1981) growing on '*C. fulvida*'.

1.4.1.2 Parasitic insects

A number of different parasitic insect species are recorded from *Ozothamnus* (Appendix 2). The most obvious of these are the gall-forming insects and mites, the first causing a swollen shoot apex surrounded by a rosette of leaves which are up to twice normal size, while the second caused a 'witches broom' type gall at the tips of a branch cluster.

Gall-forming insects

The monothalamous (single-chambered) gall-forming insects are undescribed parasitic Cecidomyiidae and Tephritidae (R. Emberson, Entomology Dept. Lincoln University pers. comm.). A number of gall midge species attack shrubs in New Zealand but they are difficult to identify. These tiny flies typically measure one to few millimetres long. They lay their eggs in the leaves or developing buds of the host. The developing larvae, which are often yellow, orange, or pink, appear during October and eat the centre of the shoot apex (Scott 1984). At the same time they cause the shoot tip to swell and the leaves to expand. The affected shoots die after the imago has emerged, though in some, an apical zone appeared to be re-established. Lush (1948 in Lamb 1960) recorded a monothalamous gall caused by a mite in '*C. fulvida*'. Gall-forming cecidomyiid larvae have been recorded from plants at Porter's Pass, Broken River, Cass, and near Otira by Molloy (1959) and Sheppard (1965). *Tephritis* sp. (Diptera: Tephritidae) has been observed by Tillyard (1926) on '*Cassinia* species'. *Tephrititis cassiniae* Mall., a Tephritidae species named after the

host plant '*Cassinia*' was described by Malloch (1931) and recorded from '*C. leptophylla*' by Harrison (1959).

'Witches brooms' in '*C. fulvida*' and '*C. vauvilliersii*' caused by a gallmite were recorded by Ramsay (1952 in Lamb 1960) and Moar (1958 in Lamb 1960) respectively. The witches-broom gall has been tentatively identified as an undescribed species of eriophyd mite (*Eriophyes sp.* (Acari: Eriophyidae)) (Moar 1958 in Lamb 1960). This mite appears to prevent internode elongation of stems on the opening of leaf buds. At the same time a multitude of similarly affected shoots is formed. The mites generally seem to occur near the base of the leaves though a few are found towards the leaf tip. These infected shoot systems usually die the following year (Sheppard 1965).

Sheppard (1965) recorded several species of mite in cavities of the stem of *Ozothamnus*. He thought that the mite secondarily invaded these cavities, as there were signs of wound tissue forming around the cavity. Dr. J. G. Sheals of the Department of Zoology, British Museum of Natural History identified these mites as having affinities with *Dolichotetranchus ancistrus*.

Seed flies

Insects eating seeds of *Ozothamnus* belong to the Anthomyiidae (seed flies) and have been recorded by Sheppard (1965) during studies on the reproductive potential of *Ozothamnus*. Sheppard (1965) and Wilton (1997) also mentioned nymphs of psyllid species, possibly similar to *Trioza acuta* (Ferris & Klyver) (Hemiptera: Psyllidaea) as seed predators. The larvae of Tephritidae species are also known to eat seeds of *Ozothamnus* (R. Emberson, Entomology Dept. Lincoln University pers. comm.).

Tachinid flies (Diptera: Tachninidaeare), the most common floral visitors of *Ozothamnus* in the Cass district according to Wilton (1997), feed on the corolla lobes and the top of the corolla tube.

Sap sucking Hemipterans

The vast majority of parasitic insects found on *Ozothamnus* are sap sucking insects such as leafhoppers (Hemiptera: Cicadellidae), lerp insects or psyllid species (Hemiptera: Psyllidae), Lygaeidae (Hemiptera) and scale insects (Hemiptera: Coccidae). The leafhoppers *Novothymbris cassinae* (Myers) and *Zygina zealandica* (Myers) have been

recorded from '*Cassinia leptophylla*' by Myers (1923). '*C. leptophylla*' is also the host for the three Lygaeidae species *Nysius* sp., *Nysius huttoni* F.B. White (wheat bug), and *Rhypodes clavicornis* (F.) (Myers 1926). *Nysius* spp. and *Rhypodes* ssp. were observed by Wilton (1997) to feed also at the top of the corolla tubes of *Ozothamnus leptophyllus*. The citrophilus mealy bug, *Pseudococcus calceolaria* (Maskell) (Hemiptera: Pseudococcidae) feeds by sucking sap from '*Cassinia*' species (Maskell 1879). So does the lerp insect *Trioza acuta* (Ferris & Klyver) (Hemiptera: Psyllidae) which was described from '*C. leptophylla*' by Ferris & Klyver (1932). *Bipuncticoris cassinianus*, a new endemic genus and species of Mirinae (Hemiptera: Miridae) for New Zealand, was described in 1995 by Eyles and Carvalho. It was found on '*Cassinia*' growing at the Black Birch Station, near Blenheim, Marlborough. The species occurs in great numbers and probably breeds on the plants (Eyles and Carvalho 1995).

Coccidiae and sooty mould

Ozothamnus plants covered with a sooty mould growing on honeydew excreted by scale insects (Coccidiae), similar in appearance to that on *Nothofagus* species and *Leptospermum*, can be seen occasionally. Several Coccidiae are described from *Ozothamnus*. Maskell (1891) described *Saissetia cassiniae* (Mask.) (as *Lecanium cassiniae*) a scale insect he found on '*C. leptophylla*' in Wellington, Wairarapa, and Hawke's Bay. He remarked: "As *Cassinia* is in many parts of this country a great nuisance and useless encumbrance, the occurrence on it of a lecanid, often in great numbers, may be considered as a satisfactory thing; but although sometimes the plants over a large area are quite blackened with the fungus induced by the "honeydew" of the insect, I cannot find that any good results have followed from its visitation" (Maskell 1891:15). Maskell (1885) described the black scale *Saissetia oleae* (Ol.) (as *Lecanium oleae*) from '*C. leptophylla*'. Another Coccidae parasitising '*Cassinia*', *Eriococcus parabilis*, found near the Desert Road in the Tongariro Nt. Park was described 1962 by Hoy. Sheppard (1965) also reported having observed '*Cassinia*' plants covered with sooty mould. *Ozothamnus* was not killed by the blight, as with *Leptospermum*, although the vigour of the infected plants was reduced (Sheppard 1965).

Lepidoptera

Ozothamnus leptophyllus is host plant for many butterflies and moths. The following looper caterpillar species (Lepidoptera: Geometridae) are recorded from *O. leptophyllus* and probably feed on the top of the corolla tubes (pers. obs.): *Pseudocoremia rudisata* (Walk.) feeding on '*C. leptophylla*' (Hudson 1928), *Pseudocoremia colpogramma* (Meyr.) feeding on '*C. vauvilliersii*' (Meyrick 1936, Hudson 1939), *Pseudocoremia dejectaria* (Walk.) feeding on '*C. vauvilliersii*' (Hudson 1939), and *Selidosema adusta* feeding on '*C. fulvida*' (Molloy 1959).

The gregarious tineid, *Heliostibes atychioides* Butler (Lepidoptera: Glyphipterygidae) has been observed on '*C. leptophylla*' by Hudson (1928). The larvae of this species web together the leaves of conifers and shrubs such as *Leptospermum scoparium* and *Kunzea ericoides*, which have small leaves. They pupate in these pendulous masses. Young shoots are similarly webbed and the tender bark eaten. The masses of webbing and leafy material frequently contain remains of other insects such as ladybirds. The webbing makes affected bushes and shrubs unsightly (Miller 1971, Scott 1984). *Ozothamnus leptophyllus* is also the host plant to the bag moth, *Liothula omnivora* Fered. (Lepidoptera: Psychidae). Feeding usually occurs at night and the larva moves about the plant carrying its case and eating foliage, and most likely floral parts. The most common host plants for the bag moth are exotic trees, *Leptospermum scoparium*, *Kunzea ericoides*, *Ozothamnus leptophyllus* and *Dracophyllum* (Hudson 1928, Scott 1984).

Two *Harmologa* species, *Harmologa sisyrana* Meyr. on '*Cassinia leptophylla*' and *Harmologa columella* Meyr. on '*C. vauvilliersii*', have been recorded by Hudson (1928, 1939). Caterpillars of the noctuid moth *Graphania homoscia* (Meyr.) (Lepidoptera: Noctuidae) and the moth *Zermizinga indocilisaria* Walk. can also be found on '*C. leptophylla*' (Hudson 1928, Clark 1935). Both of these moth species probably feed at the top of the corolla tubes (Wilton 1997, pers. obs.).

Coleoptera

An undescribed melolonthid beetle (Scarabaeidae sub-family Melolonthinae) eating leaves of *Ozothamnus leptophyllus*, and a grub thought to be the same melolonthid, eating shrub roots collected at Broken River and Cass, was mentioned by Sheppard (1965). There are

approximately 90 indigenous species of the scarab sub-family Melolonthinae in New Zealand (Scott 1984).

1.4.2 Phenology

Information about flowering and fruiting times of *Ozothamnus leptophyllus* in taxonomic literature is very sparse. Webb (1988) noted that the flowering period for this taxon is August to March, with November to February as main flowering season. Allan (1961) also stated November to February as the flowering period and remarked that fruits appear soon afterwards, occasionally until April. Cheeseman (1906 and 1925) suggested December to February as flowering time for '*Cassinia leptophylla*' and '*C. fulvida*', December to January for '*C. amoena*' and '*C. vauvilliersii*', and November to January for '*C. retorta*'. Kirk (1899) only mentioned the flowering times of '*C. retorta*' (November to January) and '*C. fulvida*' (December to February). Flowering times from December to March and fruiting times from March to April for '*C. leptophylla*' are reported in New Zealand guide books (e.g., Wilson & Galloway 1993, Wilson 1996). '*C. retorta*' seems to start flowering earlier than the other '*Cassinia*' species (Cheeseman 1906 and 1925, Kirk 1899), probably due to the milder climate of its distribution area from North Cape to 38°30'S. Salmon (1991) indicated non-flowering gaps, or pulses, during the flowering season of '*C. leptophylla*' by mentioning November to January and March and April as flowering times.

Detailed studies on the phenology of *Ozothamnus leptophyllus* in the Cass district were made by Sheppard (1965) and Wilton (1997). Sheppard's survey was made from January 1963, until January 1964 and then again in September and October 1964. Flowers began to appear at the end of September in warm sheltered localities. Flowering did not generally begin until Mid-November (in 1963) and reached a maximum in December and early January. By February, flowering began to wane although isolated individuals continued flowering until May. The early flowering in October 1964, was probably a reflection of the warm moist spring experienced (Sheppard 1965).

The aim of Wilton's study (1997) was to document the patterns of flowering phenology at the association, population, individual, capitulum, and floret levels of species

of New Zealand Gnaphalieae of the Cass district. *Ozothamnus leptophyllus* was one of the species studied in detail. The flowering periods recorded for *O. leptophyllus* were mid January to early April in 1994, early January to early April in 1995, and mid January to mid May in 1996. The individuals of *O. leptophyllus* appeared to start flowering in approximately the same order each year, with the same individuals usually flowering later each season. Non-flowering gaps, or pulses, during the flowering season were occasionally observed for some individuals of *O. leptophyllus* by Wilton. Pulsing can benefit individuals since late flowers may receive higher pollinator visitation rates or avoid high levels of predation that may occur during the main flowering period, but can also result in the abortion of capitula with the occurrence of frost later in the season (Wilton 1997).

The cluster phenology of *O. leptophyllus* seems to follow no fixed pattern (Wilton 1997). He showed clearly how the phenology of the capitulum and florets influence the level of geitonogamy and interference between male and female functions. He described how initially a very tightly-closed capitulum opens by the extension of the white-tipped inner involucral bracts above the bronze outer involucral bracts. The inner involucral bracts begin to separate and bend. One or occasionally a group of two to three florets may form the initial group to open. Subsequently floret groups of one to three open. The last floret to open usually occurs singly. The florets brown in the order in that they opened (Wilton 1997).

The florets of *O. leptophyllus* are all hermaphrodite. Male and female functions are separated temporally. The female function occurs after the male function (protandry), allowing the pollen and stigma to be presented in the same position, and preventing autogamous self-pollen from being wasted, or clogging the stigma. The stamen tube extends as soon as the corolla opens. When the pollen is presented, the corolla lobes are fully extended, and usually curl under at the tips. After pollen presentation the stamen tube begins to withdraw and the style starts elongating. By the time the stamen tube has withdrawn to the level of the corolla tube, the style arms are well separated, and slightly curled. At this point the style arms continue to curl so that they point back towards the style base. The curling of the style arms reduces interference between neighbouring florets. At the end of the female phase, the style begins to withdraw into the corolla tube and begins to brown. The corolla begins browning after the style is completely brown. When completely brown, the corolla is usually level with, or just below, the top of the pappus hairs. The

withdrawal of the floral parts when their function is complete helps to avoid the interference with the next phase of the same floret, or the function of adjacent florets (Wilton 1997).

Seed ripening appears to begin in late January, reaching a peak in March when large quantities of seed are released. Generally the seed remains in a cluster about the seedhead until it is dispersed by wind (Sheppard 1965). Both Sheppard (1965) and Wilton (1997) determined the seed set of *Ozothamnus leptophyllus* florets.

Sheppard (1965) examined 500 florets and collected only ten full seeds. After 22 days on moist filter paper one seed germinated, with a second the following day. In the subsequent 14, days only one further seed germinated. This gave a percentage germination of only 30% (or only 0.6% if all florets examined were counted). In another germination test in 1964, a percentage germination of 41.7% was found. Sheppard also determined the seed set of a range of individual of *Ozothamnus leptophyllus*. Between five and eight florets were generally found per capitulum. Generally from 16 to 30 capitula occurred in each panicle giving, on an average, 150 flowers per panicle. This, on the basis of two seeds for 100 flowers, would give about three seeds per panicle, or about 1-2 fertile seeds per panicle. Sheppard (1965) encountered a considerable loss of seed through seed predators, but also remarked that it is likely that large quantities of seed are produced because a plant may have an excess of 200 panicles, which would give over 300 fertile seeds per plant.

Wilton (1997) found a proportion of seed set by florets of *Ozothamnus* of only 9%. One explanation for the relatively low percentage of seed set given by Wilton is that *Ozothamnus leptophyllus* contains some individual capitula that function only as males, which do not set seed.

1.4.3 Pollinators

A wide variety of lepidopterans, bees, and flies visit the flowers of *Ozothamnus leptophyllus*. None of the pollinators are specialised. All of them visit a wide range of indigenous New Zealand plants as well as introduced species (Primack 1983).

Dipterans, the numerically most abundant flower visitors in New Zealand, are the most common pollinators of *Ozothamnus leptophyllus* (Primack 1983, Wilton 1997). The

most important dipteran family is the Tachinidae. Primack (1983) observed the following tachinid flies visiting '*Cassinia vauvilliersii*' in Mount Cook National Park, Arthur's Pass National Park and the Craigieburn Mountains : *Erythronychia aliena* Malloch, "*Occisor*" *versutus* Hutton, "*Peremptor*" *modica* Hutton, *Proscissio cana* Hutton, *Zealandotachina nigrifemorata* Malloch, *Zealandotachina varipes* Malloch. Tachinid flies feed from the top of the corolla tubes (see parasites) but also contact the reproductive floral structures with their mouth parts and transfer pollen (Wilton 1997). Dipteran species other than tachinid flies are also common visitors to *O. leptophyllus*. Tephritid species, like the tachinid flies, are known to feed at the top of the corolla tube. They also use the plants as courtship and mating arenas, and lay their eggs in the capitula (Wilton 1997). Hoverflies (Syrphidae) were observed to take both pollen and nectar from *O. leptophyllus* flowers (Primack 1983, Wilton 1997). Syrphidae species visiting '*Cassinia vauvilliersii*' at the above mentioned localities, and '*C. fulvida*' in the Cass district were recorded by Primack (1983), specifically *Helophilus hochstetteri* Nowicki, *Melangyna ortas* (Walker), *Melangyna novaezelandiae* (Macquart), and a unidentified *Melangyna* species. Primack (1983) also recorded a calliphorid fly (*Calliphora quadrimaculata* (Svederus)), and a unidentified species of the Muscidae from '*Cassinia fulvida*'. Some cranefly (Tipulidae) species are also known to feed on flowers of *O. leptophyllus* by poking their proboscis down the narrow tubular florets (Johns 1975).

Solitary bees (Hymenoptera: Apoidae) pollinate *O. leptophyllus*, in the same manner as hoverflies, by taking pollen and nectar, and have been observed by Primack (1983) and Wilton (1997). Coleoptera and Lepidoptera (e.g., Lycaenidae: *Lycaena salustius* (Fabricius), Nymphalidae: *Argyrophenga antipodum* Doubleday) have been also reported on *O. leptophyllus* flowers (Primack 1983, Wilton 1997).

1.4.4 Growth

Ozothamnus leptophyllus is usually described as a shrub between 30 cm and 3 m tall. The height is not only influenced by environmental factors but also varies among the formerly described '*Cassinia*' species, as does the growth habit and the general appearance of the shrub.

Wardle (1963) and Sheppard (1965) have made studies on the growth habit of *Ozothamnus leptophyllus* (as *Cassinia*). '*Cassinia vauvilliersii*' was one of the species Wardle included in his study of growth habits of New Zealand subalpine shrubs and trees. Both researchers provided information about the aspects of the vegetative growth such as indications of periodicity of growth, the appearance of annual growth rings, nature of apical buds, annual cycle of growth in length, persistence of leaves, growth rates and growth form and stature. Wardle (1963) found that '*Cassinia vauvilliersii*' has very marked growth rings, and was able to show that each ring corresponds to one year's growth. Sheppard (1965) did not see growth rings clearly in '*Cassinia fulvida*' samples until the branches or stems reached an estimated eight years. Wilton (1997) observed growth rings in *Ozothamnus leptophyllus* stems (refer to 1.4.6). The overwintering apical buds of '*Cassinia fulvida*' are "unspecialised". They are protected only by developing foliage and not by special protective structures as in most trees and shrubs of the New Zealand mountains (Wardle 1963). The amount of growth since the last period of winter rest is indicated by the small size of the leaves that are formed close to the resting period (Wardle 1963). Sheppard (1965) found it difficult to distinguish between spring and summer leaves because the leaves of '*Cassinia fulvida*' are smaller than those of '*C. vauvilliersii*', examined by Wardle. Using leaf sizes as a base for measurement of stem elongation (i.e. up the stem from small spring leaves to larger summer leaves) was not entirely successful for Sheppard (1965) as the leaves, which last about one year, are shed throughout the year with maximum fall in late winter and spring. This would coincide to some degree with the period of maximum stem elongation reported by Wardle (1963). Wardle (1963) also had difficulties in obtaining reliable measurements of shoot growth although he reported values of from 0.8-6 cm per annum for '*Cassinia vauvilliersii*'. Shoots measured at Cass by Sheppard (1965) appeared to extend this range to about 10 cm per annum.

Attempts were made by Sheppard (1965) to trace the rooting systems of '*Cassinia fulvida*'. Plants sampled from the Cass Fan each showed a shallow rooting system, rarely exceeding in depth 1/4 the height of the shrub. Lateral spread of the roots was also limited although several roots extended to considerable distances laterally. These findings were confirmed when shrubs in 'wash-outs' in the Broken River basin were examined. In a small shrub uprooted at Cass, traces of a taproot were seen, though generally the shrubs had a tangled rooting system with many roots. Seedlings that germinated had a single root that

gave out laterals, so it seems likely that the root system is modified as the plant becomes older (Sheppard 1965).

Ozothamnus leptophyllus has high regenerative powers after being damaged or burned. Sheppard (1965) observed that '*Cassinia*' shrubs showed basal renascence following grass fire. After very fierce burning (where the soil humus was destroyed) the number of shrubs showing signs of renascence dropped sharply until no regeneration was seen on sites where the fire had been most severe. Sheppard studied areas where fires occurred in 1947, 1959 and 1963 in the Cass district and came to the conclusion that '*Cassinia*' can regenerate after fire providing the fire is 'not too hot' and does not burn the soil. Occasionally it may survive a comparatively hot fire if the soil did not reach a temperature above the limit of tolerance of the plant. If regeneration does occur after fire, it is probably because the fire moved rapidly, so that the ground did not heat up excessively. The spread of *Ozothamnus leptophyllus* seems to be favoured by high grazing pressures. These high grazing pressures tend to keep the vegetation open, allowing ample bare ground for *O. leptophyllus* to develop (Sheppard 1965). *O. leptophyllus* favours an open habitat (refer to 1.3).

1.4.5 Cytology

Somatic chromosome counts for *Ozothamnus leptophyllus* were made by Dawson and Beuzenberg (2000). They sampled five geographically and morphologically diverse representatives of *O. leptophyllus* (including an entity listed in Druce (1993) and probably a representative of *Cassinia vauvilliersii* var. *albida*). Even though they were able to prepare slides with numerous, well-stained cells, Dawson and Beuzenberg had difficulty determining the precise number of chromosomes for *Ozothamnus leptophyllus*. Some of the cells had $2n = 26$, while others in the same preparation yielded $2n = 28$. Unable to provide a definitive result, the researchers gave two possibilities to explain this observation. One explanation they provided was that the difference might relate to stages of mitosis examined. One chromosome pair at the early metaphase may not be fully condensed, giving the impression of $2n = 26$ although the true number is $2n = 28$. If $2n = 26$ is correct, it is possible that at full metaphase a chromosome pair with a large centric gap

may be confused with two chromosome pairs, giving the impression of $2n = 28$. A further possibility is the presence of two unstable B-chromosomes that may vary from cell to cell (Dawson & Beuzenberg 2000). The researchers suggested that supporting meiotic counts should be made to provide more definitive results.

1.4.6 Anatomy

Betts (1920b) gave a description of the stem and leaf anatomy of '*Cassinia vauvilliersii* var. *rubra*'. She described epidermis, cortex, pericycle fibres, phloem, xylem and pith.

More detailed studies on the stem anatomy have been undertaken by Wilton (1997). He described sixty-eight stem anatomy characters for *Ozothamnus leptophyllus*. Wilton used transverse sections of the primary stem near the apex, mature primary stem, and mature secondary stem. The following summarises the observations made by Wilton:
The pith of the young stem is composed of thin-walled parenchymatous cells. In the mature secondary stem these pith cells have only collenchymatous thickening and no lignified cells like all the other Gnaphalieae species included in Wilton's study. The pith end walls have a fibrous appearance. A ring of collateral bundles, which gives rise to numerous leaf traces, surrounds the pith.

A mix of thickened primary elements and parenchymatous cells forms the xylem in the young stem. Secondary phloem and xylem are produced by a single vascular cambium that develops between the primary xylem and phloem. The vessels in the mature stem are arranged in clumped aggregations of eight or less vessels. The secondary xylem is composed entirely of lignified elements.

Growth rings occur in the secondary xylem, delimited by the variation in the radial width of the imperforate tracheary elements. The tracheary elements reduce in width gradually through the growing season, but the difference in width between the late wood of one growth ring and the early wood of the next is significant. Growth rings are also marked by changes in vessel diameter and abundance. In the pericyclic region, sclerenchymatous fibres develop. Sclerenchymatous cells occur as a few thick-walled cells at points around the bundle sheath. *O. leptophyllus* has prominent multiserate rays.

The stele (i.e. the vascular tissue, the ground tissue between the vascular bundles and the pith) is surrounded by an endodermis. The enodermis appears to undergo periclinal divisions in the very young stem near the stem apex, sometimes making it difficult to detect in the youngest sections. In the mature stem the endodermis is normally conspicuous due to thickenings of the cell walls. Endodermis cells with all walls thickened are especially characteristic for *Ozothamnus* and *Cassinia*. *O. leptophyllus* also has strongly birefringent endodermis walls.

The cortex in young *O. leptophyllus* stems is composed of generally thin-walled parenchymatous cells with large intercellular spaces in the outer cortex. Spaces develop near the surface of the stem, but are separated from the epidermis by two layers of cortex cells. The cortex cells appear to show collenchymatous type thickenings, especially in the region of leaf sheath development. The cortex cells contain chloroplasts; especially the outermost cells and prominently raised stomata can be found in the epidermis of the young stem.

A dense layer of uniserate and biserate hairs with swollen terminal cells is also present. The development of a periderm in the pericyclic region of the phloem in the mature stem results in the loss of the epidermis, the cortex, and the endodermis. The periderm is composed of large pale staining cells, indicating the presence of suberin and lignin. The periderm gradually becomes more deeply seated, so that in the fully mature stem it is located in the outer region of the phloem. The phellogen appears to produce cells only to the outside.

Breitwieser (1993) included two representatives of New Zealand's *Ozothamnus* ('*Cassinia fulvida*' and '*C. leptophylla*') in her study on the leaf anatomy of 45 species of Gnaphalieae. The following observations on the leaf anatomy of '*Cassinia fulvida*' and '*C. leptophylla*' were made by Breitwieser (1993).

The lamina is dorsiventral, 300 µm thick in '*C. fulvida*' and 200 µm thick in '*C. leptophylla*' with a cuticle less than 5 µm thick. The epidermis is composed of regular isodiametric or oval cells at the adaxial surface and irregularly shaped cells at the abaxial side. The stomata are confined to the abaxial surface and raised above the normal epidermis cells. The mesophyll is clearly differentiated into pallisade and spongy parenchyma.

The palisade tissue is confined to the adaxial side, compactly arranged, and 80-190 µm thick in '*C. fulvida*' and 100 µm thick in '*C. leptophylla*'. The rod-shaped palisade cells are arranged in 2-6 rows in '*C. fulvida*' and in 2-3 rows in '*C. leptophylla*'. The size of the palisade cells varies between '*C. fulvida*' and '*C. leptophylla*'. They are 20-80 µm long and 10-20 µm wide in '*C. fulvida*' and 40-50 µm long and 20-30 µm wide in '*C. leptophylla*'. The spongy tissue is loosely arranged, 70-100 µm thick in '*C. fulvida*' and 80 µm thick in '*C. leptophylla*'. The 10-20 µm long cells of the spongy tissue are elongated and parallel to the leaf surface. The leaf-margin is rounded with palisade cells continuous around the periphery of the leaf. The midrib is protruding 100-150 µm abaxially, but nearly level adaxially. The midvein, 70 µm in diameter in both of the examined "species", is much closer to the abaxial than to the adaxial surface and surrounded by a single-layered parenchymatous bundle-sheath. No sclerenchyma caps are present.

The lateral ribs are not protruding and the major veins are 20 µm in diameter and much closer to the abaxial than to the adaxial surface.

Prior to Breitwieser's observations, Betts (1920b) had mentioned the presence of hairs with two or three small cells at the base and a long cell at the end on the upper but mainly at the lower epidermis.

1.4.7 Studies of flavonoid compounds

Free flavonoid aglycones in form of quasi-crystalline deposits on the abaxial leaf surface and young branchlets have been encountered in *Ozothamnus leptophyllus* (Wollenweber 1984).

The major components, isolated from *O. leptophyllus* are 2',6'-dihydroxy-4'-methoxychalcone, 2',4',6'-trihydroxychalcone, 2',4'-dihydroxy-4'-methoxydihydrochalcone and 1'-(5'-acetyl-2'hydroxyphenyl)-3-methyl-2-buten-1-one (Wood et. al 1999). Besides these components Wood (1992) identified the artefact 5-Hydroxy-7-methoxyflavanone (pinostrobin). Reid and Bohm (1994) isolated some additional exudate flavonoids from several *O. leptophyllus* specimens: 2'-Hydroxy-4'-methoxychalcone; 2',4',3,4-Tetrahydroxycgalcone; 5,7-Dihydroxyflavanone (pinocembrin); 5,7,3',4'-

Tetrahydroxyflavanone (eriodictyol); Quercetin-3-*O*-methyl ether; Quercetin-7-*O*-methyl ether.

Differences in exudate flavonoid profiles and concentrations were found between the material from several populations (Wood 1992, Breitwieser & Ward 1993, Reid & Bohm 1994). Breitwieser and Ward (1993) found variation in the semi-quantitative distribution of leaf flavonoids between '*Cassinia fulvida*' and '*C. leptophylla*'. The concentration of four different compounds was in '*C. fulvida*' "high" compared to a low concentration in '*C. leptophylla*'. No quasi-crystalline deposits could be found by Wood (1992) in '*Cassinia leptophylla*'. Reid and Bohm (1994) isolated just a single exudate flavonoid, 5,7-Dihydroxyflavanone (pinocembrin) from leaves of '*Cassinia leptophylla*'. The chalcones which are responsible for the yellow colour of leaves and branchlets of '*Cassinia fulvida*' are absent in the flavonoid profile of '*C. leptophylla*', which is meant to have a greyish-white tomentum with no traces of yellow. Reid and Bohm (1994) could show that flavonoid profiles were constant within a population.

Wood et al. (1999) tested the biological activity of the compounds found in *Ozothamnus leptophyllus*. Two of the compounds, 2',4',6'-trihydroxychalcone and 2',4'-dihydroxy-4'-methoxydihydrochalcone showed anti-viral activity. 2',6'-dihydroxy-4'-methoxydihydrochalcone was weakly antimicrobial and active against a virus (*Herpes simplex*).

The observations of flavonoid variation among populations of *O. leptophyllus* (Wood 1992, Breitwieser & Ward 1993, Reid & Bohm 1994) provided an additional set of data that could be used to argue taxonomic recognition of distinct "forms". Further detailed work on flavonoids would be of considerable interest.

1.4.8 Cultivation and propagation *Ozothamnus leptophyllus*

Ozothamnus leptophyllus is not only a major component of New Zealand's natural vegetation but is also increasingly popular for revegetation, enhancing local habitats and for ornamental purposes when landscaping. It is particularly suitable for growing in dry exposed places and especially for providing quick shelter for other shrubs, and will tolerate a wide range of soil types (Matthews 1979, Metcalf 2000). *O. leptophyllus* has a high salt

and wind tolerance, which makes it an ideal plant for sand dune restoration. It is, for example, used in Christchurch for the re-establishment of coastal bush behind salt marsh areas at Ferrymead and the re-development of the Southshore Spit (Management Plans & Landscape Plans of the Christchurch City Council, <http://www.ccc.govt.nz/PARKS/ ManagementPlans>). It is also frequently used for slope stabilisation and revegetating stream banks and disturbed sites which have had the topsoil removed (Bay of Plenty Regional Council Land Management (Environment B·O·P), <envbop.govt.nz/www/ Responsibilities/LandManagement/SoilConservation/RetirementAreas.htm>).

Ozothamnus leptophyllus still does not rank among the elite of the New Zealand's cultivated shrubs but its popularity as a garden plant is increasing. Nurseries specialising in New Zealand natives frequently provide *O. leptophyllus* for sale (pers. observ.). Its high frost tolerance (up to -15°C, according to the Durham/UK University Botanic Garden Plant Index: <http://www.dur.ac.uk/~deb0www/dubg/cass.html>) and low maintenance requirement make it a popular cultivated plant in many overseas countries. Botanical gardens with *O. leptophyllus* in their plant collections are located in the USA (e.g. Washington Park Arboretum, University of Washington, Seattle, USA, depts.washington. edu/wpa/highcountry.htm), Britain (e.g. Durham University Botanic Garden, Durham City, UK, <http://www.dur.ac.uk/~deb0www/dubg/bghomep.html>) and other north European countries (e.g. Forstbotanisk Have i Århus, Denmark; Skógrøkt Landsins, Faeroe Islands; Universitetet i Bergen, Arboretet og Botanisk Hage, Milde, Norway (The NGB Horticultural Network (HCN), <http://www.ngb.se/Databases/HCN>)). Overseas nurseries sell *O. leptophyllus* (usually advertised as '*Cassinia* species' or 'varieties') as a garden plant as seen on stock lists available on the internet (e.g. Pine Lodge Gardens, Kingston, WA, USA, <http://www.pine-lodge.co.uk/Price%20List/prindex.htm>; Bradley Nursery & Gardens, Wylam, UK, <http://www.bradleygardens.co.uk/pages/catalogue/Evergreen% 20shrub/ 8471.html>; Rumsey Gardens, Hants, UK, <http://www.my.genie.co.uk/dgiles/ rg_pages/shrubs.html>; Arboretum Trompenburg Rotterdam, Netherland, <http://www.esveld.nl/dialijst/dialistC.html>). *O. leptophyllus* is even exported as fresh cut flower (Pacific Blooms Ltd., <http://www. pacificblooms.com/PBL/product_list.htm>).

Ozothamnus leptophyllus is particularly easy to grow (Metcalf 2000) and can be quickly propagated by hardwood and semi-hardwood cuttings (Bryant 1992). Cuttings can be made from side shoots, 4 to 6 cm long, which are detached along with a thin 'heel' of old wood. Bryant (1992) gives a strike time of 25-50 days and a strike rate of 40-80%. He also suggests placing the cuttings under mist or fog to decrease the time to strike. Collection of cuttings in winter (at the end of July) provides the best results, especially in combination with wounding (the removing of a thin slice of bark for about 2.5 cm from the base to expose the cambium without cutting deeply into wood) (Follet & Foggo 1981). Cuttings from younger stock plants or those containing some juvenile tissue generally root more readily than those from older plants (Follet & Foggo 1981).

Ozothamnus leptophyllus is fast growing. When grown in the garden it should be given an annual pruning immediately after flowering to keep it neat and compact and to prevent the bushes from becoming leggy. The leading growth may be pruned back quite hard and all others lightly pruned (Metcalf 2000).

1.5 Methodological scope

The research for this thesis lies at the interface of taxonomy and biosystematics, using the term taxonomy in the sense of naming, describing, identifying, and classifying and biosystematics in the sense of investigating natural variation. When used in this sense, taxonomy is also known as orthodox or classical taxonomy (Heslop-Harrison 1960, Stuessy 1990, Briggs & Walters 1997). Biosystematists attempt to understand the evolutionary forces that work together in producing taxa and contribute to an understanding of populations and the processes by which they have changed and are changing, but do not dictate the taxonomic decision made in the light of this information (Raven 1974). Stace (1989) recommends the distinction between biosystematics and classical taxonomy but emphasises that these two fields are not separate and opposing, but rather are closely interacting.

Taxonomy is a dynamic discipline, and classifications are constantly changing and expanding. Our knowledge of the structure and function of organisms is continually being

increased as new discoveries are made. As our pool of knowledge expands new evidence becomes apparent and our perception of the taxa and their relationships to one another also changes (Stuessy 1990). Over the last twenty years the fields of molecular genetics and chemotaxonomy have flourished, producing a wealth of data (Stuessy 1990: 313, 329, Soltis et al. 1992, Thorne 2000), providing additional information and giving new insights into relationships between taxa previously classified only by morphology, anatomy, and cytology. Beside morphological characters, molecular data in the form of AFLP (amplified fragment length polymorphism) fingerprints will be employed in this study to determine if there are any distinct entities within the *Ozothamnus leptophyllus* species complex and to clarify the taxonomic status for taxonomically recognisable entities.

The methods of data analysis as well as the type of data used for classification are often influenced by dominant trends in thinking; at any one time a certain technique may be far more fashionable than any other. As the methods used to form a classification change and emphasis is moved to new considerations the classification itself may undergo change. This is clearly illustrated in the contrast between phenetic and phylogenetic classification (Stuessy 1990). In the late 1950s-1960s some biologists assumed that the phenetic classification systems would be the best way to organise and catalogue biological diversity. Central tenets of phenetics are that taxonomic groups are based on the degree of overall similarity, which is calculated by comparing all the units of study (OTUs) over a large number of equally weighted characters. Some pheneticists argued that it is impossible to know with any degree of certainty the phylogeny of a taxon, and that the most stable means of classification is the analysis of the degree of overall similarity between any two taxa (Davis & Heywood 1963: xviii; Sokal and Sneath 1963: 7).

Numerical techniques were commonly used during the 1960s and '70s, particularly with the increasing utilisation of computers to process large amounts of data (Duncan & Baum 1981). However, during the late 1970s and 1980s, cladistic analysis began to have a marked impact. Phylogenetic taxonomists, including the advocates of cladistic techniques, maintained that only by tracing the evolutionary pathways may the relationships between related taxa be known. Nevertheless numerical taxonomy remains an important basis for classification at lower levels, and phenetic methods are still regarded as a relatively standard technique in molecular systematics, for use with results from electrophoresis (allele matching), DNA hybridisation (melting points), and nucleotide or amino acid

sequence analysis. A phenetic approach using analysis of morphological and molecular data was chosen to detect groups within *Ozothamnus leptophyllus*, leading to a new classification for this complex.

Taxonomically, the genus *Ozothamnus* in New Zealand presents a complex problem. Intermediate forms have been recorded in many instances from regions of "species" overlap. *O. leptophyllus* "species" appear to hybridise freely (Cockayne 1928) causing difficulty in delimitation. Thus hybridisation and regional introgression may have contributed to variation patterns. When Webb (1988) combined the five '*Cassinia*' species into a single species, he obscured marked morphological differences found at the extremes of possibly continuous variation. However it would be inappropriate to obscure continuity by creating what are no more than convenient units. Fortunately these two extremes are not the only possibilities (e.g., Fisher 1965).

This research aims to define natural groups and to produce a stable system of classification for the *Ozothamnus leptophyllus* species complex by assessing variation at different levels within and among populations based on experimental cultivation and numerical phenetic analyses of morphological and genetic data. A major goal of this study is to determine the most appropriate taxonomic status for recognisable entities, should such entities be found to exist. This research will also meet the need for stable scientific names for any taxonomically recognisable entities. In the event of distinct taxa being found to be present, descriptions of these taxa will be provided and a key prepared to facilitate their identification.

Chapter 2

Numerical analysis using morphological characters

2.1 Introduction

Taxonomic characters

Taxonomic evidence can be gathered from a wide variety of sources, but any data which show differences between taxa may be of taxonomic significance (Stace 1989). Attributes of organisms for any study of diversity are called systematic characters (Davis & Heywood 1963, Stuessy 1990). A taxonomic character is "a feature of an organism that is divisible into at least two conditions (or states) and that is used for constructing classification and associated activities (principally identification)" (Stuessy 1990: 27). The wide array of possible taxonomic characters makes it necessary to be highly selective in the characters that are chosen for study.

Morphological characters

The features of external form or appearance have been, and still are, the type of data most often used in plant classifications (Stuessy 1990). These exomorphic characters find practical use in keys and descriptions and have been used for far longer than any other source of taxonomic evidence such as comparative anatomy, embryology, palynology, cytogenetics, chemistry or molecular data.

Morphology currently provides most of the characters used in constructing taxonomic systems (Davis & Heywood 1963, Jones & Luchsinger 1986, Stuessy 1990, Judd et al. 1999). Most of the taxa recognised today have been delimited on the basis of morphological resemblance - those that look more like each other being placed in the same taxon. We still usually take this as a starting point for classification, largely because we are visually oriented organisms. We can quickly assess, and describe, things that we can see. It is easy, relatively quick, and inexpensive (often an important consideration) to assess morphological similarity. Most species can be identified from morphological characters.

Taxonomically informative morphological characters may be found in leaves,

stems, roots, flowers, fruits and seeds. Morphology provides one of the best mirrors of genetic and evolutionary relationships, provides clues to the way in which the plants have adapted to their environment and can be seen as the foundation for solving taxonomic problems (Stuessy 1990). Two basic types of morphological data were distinguished by Stuessy (1990): macromorphological and micromorphological. The plant characters observed most commonly in the herbarium are macromorphological, i.e. they can be detected readily by external examination with the naked eye, hand lens or dissecting microscope. Such macromorphological features are commonly used in the keys provided in Floras (e.g., Allan 1961, Webb et al. 1988). Micromorphological characters are those seen only with compound microscopes or with the scanning electron microscope (SEM). Characters of minute structures are for example of particular importance in taxonomic discrimination in Compositae (King & Robinson 1970) and include cellular details of corollas, stamens, styles and achenes, pollen morphology, leaf indumentum and leaf venation.

Within the Compositae, morphology has been and still is a major source of taxonomic data. The taxonomy of New Zealand *Ozothamnus* is traditionally based on macromorphological characters but micromorphology has also been used. For example Breitwieser and Ward (1997) transferred *Cassinia leptophylla* to *Ozothamnus* based on macro- and micromorphological evidence.

Morphological characters of '*Cassinia*' in New Zealand

The first descriptions of '*Cassinia*' species and varieties in New Zealand (Forster 1786; Brown 1817; Cunningham ex de Candolle 1837; Hooker 1847,1864; Buchanan 1887; Colenso 1890; Cheeseman 1897; Kirk 1899; Cheeseman 1906; Cockayne 1906; Cockayne & Allan 1926b; Allan 1961) contain macromorphological characters of the habit, leaves, inflorescence and florets. The characters found in the keys of New Zealand Floras (Hooker 1853, 1864; Kirk 1899; Cheeseman 1906, 1925; Allan 1961; Webb 1988) to distinguish the different taxa are the number of receptacle scales among the florets and the leaf size, shape and tomentum. A comparison of the descriptions of the '*Cassinia*' species in the different Floras is presented in Table 2.1.

Table 2.1 Descriptions of *Ozothamnus leptophyllus* in New Zealand Floras.

		Allan (1961)	Cheeseman (1925 and 1906)	Kirk (1899)	Hooker (1864 and 1853)	Webb (1988)
C. retorta A.Cunn. ex DC.	habit	Shrub up to 5 m tall, branchlets stout, white-tomentose.	Shrub 1.2-4.6 m high, much or sparingly branched heath-like; branches stout, spreading, clothed with white tomentum.	Shrub 1.5-3.7 m high, sparingly or densely branched; branches clothed with white tomentum.	Shrub 3.05-4.6 m high; branches and leaves below covered with white tomentum, not glutinous.	*Cassinia leptophylla* (G.Forst.) R.Br Shrub < 0.5-5 m tall. Stems densely tomentose and sometimes glandular, but without long straight hairs. Lvs 1.5-10-(15) x 1-3-(4) mm; from almost glabrous to moderately clothed in white tomentum on upper surface, with dense white tomentum on lower surface and sometimes with sparse to dense covering of glandular hairs giving a yellow appearance to lf, apetiolate, usually oblong to narrow-obovate or elliptic, rarely linear or ± obtriangular, slightly revolute but lower surface not generally obscured. Capitula 1-4 mm long, in small, dense, rounded corymbs; Involucral bracts sparsely to moderately hairy especially on stereome of outer bracts, mostly translucent, sometimes tinged pink or red in bud, often the inner bracts with milky white radiating lamina; florets 4-5-(25) per capitulum; receptacular scales few to many or 0, with white radiating lamina slightly exceeding corollas; achenes glabrous or with scattered antrose hairs, square in section and slightly narrowed to base, 1-1.7 mm long; pappus denticulate, scarcely to distinctly thickened at apex.
	vegetative	Lvs 2-5 x 1-2 mm, c. linear-oblong to narrow-obovate, lamina rather strongly recurved from erect petiole, coriac., becoming glab. above, beneath clad in dense white tomentum, (sts yellowish), margins revolute.	Lvs 3.2-5.1 mm long, numerous, crowded, spreading and recurved, linear-obovate or linear oblong or oblong-obovate, obtuse, narrowed into a very short petiole, coriac., glab or hoary above, beneath clothed with dense white tomentum; margins recurved.	Lvs 4.2-5.1 mm long, close-set, linear-obovate or linear-oblong, obtuse, coriac., never glutinous, narrowed into a short petiole which is closely appressed to the branch; midrib obvious beneath, margins recurved.	Lvs 4.2 mm long, close-set, spreading or recurved, linear-obovate of linear-oblong, obtuse, opaque above, margins recurved.	
	floral	Capitula 3-10 or more in small corymbs, on short pedicels; phyll. in 3-4 series, tomentose on back, inner with short white tips; forming a turbinate involucre 4-7 mm long, up to 9 mm diam.; florets 10-20; scales of receptacle ∞ with erect white tips; achenes c. 1 mm long, glab.; pappus-hairs up to 3.5 mm long, slender, hardly or not thickened at tips.	Capitula 6.4 mm long; numerous, in small terminal corymbs, shortly pedicelled, turbinate; involucral bracts in several series; the outer shorter, ovate-oblong, tomentose; inner linear-oblong, with short white obtuse radiating tips; florets 6-20; receptacle with many white-tipped scales similar to the inner involucral bracts; achenes glabrous, striate; pappus-hairs slender.	Capitula 3-8, in terminal corymbs; pedicels stout; involucres turbinate; involucral bracts ovate or ovate-oblong, the outer pubescent or cottony; florets about 8; receptacle palaeceous; achenes faintly striate, glabrous.	Capitula 6.4 mm long; 1-8 together, turbinate, shortly pedicelled, involucral scales with white tomentum; florets 6-8.	
C. leptophylla (G.Forst.) R.Br	habit	Shrub up to 2 m tall; branchlets slender, greyish-tomentose.	Much like *C. retorta* in habit and general appearance, but branches more slender.	Similar to *C. retorta*, but smaller in all parts, with more slender branches.	Shrub like *C. retorta* in habit and pubescence, also glutinous, but more slender. Very variable in size: 3.05-4.9 m. high and woodiness of the stems and branches, which are covered with white down.	
	vegetative	Lvs 2-4 x 1-2.5 mm, narrow-linear to linear-spathulate, lamina erect to spreading from appressed short petiole, coriac., becoming glab. above, beneath clad in white tomentum, margins slightly revolute.	Lvs 2.2-3.2 mm long, smaller, crowded, erect or spreading or recurved, narrow-linear of linear-spathulate, obtuse, coriac., glab. above, clothed with white or yellowish tomentum beneath; margins recurved.	Lvs 1.7-2.5 mm long, erect, spreading or recurved, narrow-linear or narrow linear-spathulate, obtuse, margins recurved, clothed with appressed white tomentum beneath.	Lvs 2.2-2.5 mm long, 1.3 mm broad, erect or spreading, rarely recurved, narrow linear, glab. above and more or less shining. Lvs small, heath-like, very numerous, uniform in size, crowded on slender branches, spreading or recurved, linear, blunt, hoary on both sides or below only, with recurved margins.	
	floral	Capitula ∞ in small dense corymbs pedicels short; phyll. in 3-4 series, outer glab. to pubescent-ciliate, inner with short white radiating tips; forming a narrow-turbinate involucre 3-4 mm long; scales of receptacle ∞, white tipped; achenes c. 1mm long, glab. or nearly so; pappus-hairs up to 4mm long, slender, slightly thickened at tips.	Capitula 4.2 mm long; numerous, in small terminal corymbs, very shortly pedicelled, narrow-turbinate, involucral bracts in several series; the outer broader and shorter, glabrous or nearly so; inner linear, obtuse, with short white radiating tips; florets 6-12; receptacle with white-tipped scales subtending the florets; achene and pappus as in *C. retorta*.	Capitula 4.2-6.4 mm long, numerous, in small terminal corymbs; pedicels very short; involucres turbinate; involucral bracts few, ovate or broadly oblong glabrate or rarely pubescent; florets 6-10.	Capitula 4.2-6.4 mm long, obconical, numerous, in terminal hemispherical corymbs, involucres longer than the leaves, imbricate, narrow turbinate or tubular; involucral scales few, scarious, glabrate, shining; florets 8-10; achenes smooth.	

Table 2.1 continued

		Allan (1961)	Cheeseman (1925 and 1906)	Kirk (1899)	Hooker (1864 and 1853)	Webb (1988)
C. amoena Cheeseman	habit	Shrub hardly up to 1 m tall; branchlets stout, greyish-tomentose.	Shrub 0.3-0.6 m high, small round-topped densely branched; branches stout, furrowed, the younger ones clothed with white greyish-white tomentum.	Shrub 0.3-0.6 m, densely branched; the younger branches clothed with greyish tomentum.	-	*Cassinia leptophylla* (G.Forst.) R.Br
	vegetative	Lvs ± 6-8 x 2-3 mm, finally spreading, narrow linear-obovate to narrow-spathulate on short erect petioles, coriac., glab. above when mature, densely white-tomentose below, margins slightly revolute.	Lvs 6.4-16.9 mm long, close-set, spreading or suberect, narrow linear-obovate or linear-spathulate, obtuse, narrowed into a short petiole, coriac., glab. above, clothed with dense white tomentum beneath; margins recurved.	Lvs 6.4-16.9 mm long, close-set, spreading or ascending, narrow linear-obovate or linear-spathulate, obtuse, clothed with white tomentum beneath, glabrous above; narrowed into a short petiole, margins recurved.	-	
	floral	Capitula ∞, in close corymbs, pedicels short. Phyll. in 3-4 series, outer tomentose to pubescent on back, inner with white radiating tips; forming a narrow-turbinate involucre 4-5 mm long; florets 4-6; scales of receptacle absent or up to 2, white-tipped; achenes c. 1mm long, pubescent to silky-hairy; pappus-hairs up to 3.5 mm long, slightly thickened at tips.	Capitula 5.1-6.4 mm long; numerous, in rounded terminal corymbs, narrow-turbinate, shortly pedicelled, involucral bracts in several series; the outer shorter, ovate-oblong, obtuse, tomentose; the inner linear-oblong, with short white radiating tips florets few, 4-6; scales of the receptacle usually absent or rarely 1 or 2 present; achene silky, with a thickened areole at the base; pappus-hairs thickened at the tips.	Capitula numerous, in crowded terminal hemispherical corymbs; pedicels short; involucres narrow, turbinate; involucral bracts narrow, the outer ovate-oblong, pubescent, the inner oblong, glabrous, membranous, with short radiating tips; florets 5; scales of receptacle few or 0.	-	
C. fulvida Hook.f.	habit	Shrub up to 2 m tall; branchlets slender, glutinous, clad in fulvous tomentum.	Shrub 0.6-1.8 m high, slender, erect, much-branched; branches glutinous, clothed with fulvous tomentum.	Shrub 0.6-1.5 m high, erect, much branched, rather slender, glutinous; branches clothed with subviscid tomentum.	Shrub with the habit, etc., of *C. leptophylla*, but glutinous, with foliage larger and tomentum fulvous, branches covered with subviscid tomentum.	
	vegetative	Lvs 4-8 x c. 1 mm, ± glutinous when young, becoming glab. above, retaining fulvous tomentum below; linear to linear-spathulate, coriac., spreading when mature from erect petiole, margins slightly revolute.	Lvs 4.2-8.5 mm long, close-set, spreading or suberect; linear-spathulate or linear-obovate, obtuse, narrowed to the base, coriac., glab. and subviscid above, beneath clothed with fulvous tomentum; margins recurved.	Lvs 4.2-8.5 mm long, spreading or ascending, sessile, linear or narrow linear-spathulate or linear-obovate, obtuse, clothed with fulvous tomentum, glutinous above, midrib obvious below, margins slightly recurved.	Lvs 4.2-6.4 mm long, spreading, linear, obtuse, fulvous below, more or less shining and glutinous above, margins recurved.	
	floral	Capitula ∞, c. 35 per corymb; phyll. in 3-4 series, outer pubescent to glab., inner with white radiating tips; forming cylindric to very narrow-turbinate involucre up to 5 mm long; florets 5-10 per capitulum; scales of receptacle absent or 1-2; achenes hardly 1 mm long, pubescent to glab.; pappus-hairs up to 3.5 mm long, slightly thickened at tips or not.	Capitula 5.1 mm long; very numerous, in terminal round corymbs, shortly pedicelled, cylindrical, involucral bracts few, in several series; outer shorter, pubescent to glabrate; inner with short white radiating tips. florets few, 5-8; scales among the florets wanting or 1 or 2 only; achenes pubescent; pappus-hairs few, thickened above.	Capitula 4.2-6.4 mm long, very numerous, in terminal simple or compound corymbs, cylindric or oblong; involucres cylindric, pubescent or glabrate; florets 6-10; scales among the florets few or 0.	Capitula 4.2-6.4 mm long, very numerous, in terminal corymbs, involucral scales pubescent or glabrate; florets 4-5; scales amongst the florets few or 0.	

Table 2.1 continued

		Allan (1961)	Cheeseman (1925 and 1906)	Kirk (1899)	Hooker (1864 and 1853)	Webb (1988)
C. vauvilliersii (Hombr. et Jacq.)	habit	Shrub up to 3 m tall; branchlets rather stout, furrowed, ± glutinous, clad in fulvous tomentum.	Shrub 0.6-1.8 m high, erect closely branched; branches stout, erect or spreading, often glutinous, grooved, and with the leaves beneath densely clothed with fulvous or whitish tomentum.	Shrub 0.6-2.4 m high, erect, much branched; branches fastigiate or spreading, stout, clothed with viscid fulvous or yellowish tomentum, grooved.	Shrub 0.6(1.8)-3 m high, erect, dense, fastigiately-branched; branches furrowed and covered with a closely appressed, buff-coloured tomentum, which also clothes the under surface of the leaves (fulvous tomentum).	*Cassinia leptophylla* (G.Forst.) R.Br
	vegetative	Lvs 5-12 x 2-3 mm, finally spreading from erect petioles, coriac., becoming glab. above, clad in fulvous tomentum below, linear-spathulate to narrow oblong-ovate, midrib prominent below, margins slightly revolute.	Lvs 6.4-8.5 mm long or more, numerous, close-set, erect or spreading, linear-obovate or linear-oblong, obtuse, narrowed into a short broad petiole or sessile, coriac., glab. and usually glutinous above, fulvous or white and strongly costate beneath; margins recurved.	Lvs 6.4-8.5 mm long, spreading or erect, linear-obovate or oblong-spathulate, coriac., obtuse, narrowed into a short broad petiole or sessile, glab. or glutinous above, clothed below with fulvous tomentum; margins flat or recurved.	Lvs 6.4-8.5(12.7) mm long, erect or patent, coriac., spreading or recurved, decurrent on the stem, linear-obovate, oblong or spathulate, obtuse (blunt) or retuse, costate below, opaque or shining and generally glutinous above, margins recurved.	
	floral	Capitula 10-20 or more, in dense corymbs, pedicels short; phyll. in 3-4 series, outer tomentose on back, inner with white radiating tips; forming a turbinate involucre c. 4 mm long; florets 8-15; scales of receptacle ∞, white-tipped; achenes hardly 1 mm long, glab. to sparsely pubescent; pappus-hairs up to 4.5 mm long, distinctly thickened at tips.	Capitula 5.1-6.4 mm long; very numerous, in terminal rounded corymbs, shortly pedicelled, turbinate, involucral bracts in several series; the outer reddish towards the tips; inner linear-oblong, with short white obtuse radiating tips; florets 8-15; scales among the florets numerous; pappus-hairs thickened at the tips.	Capitula numerous, in terminal globose corymbs; pedicels short; involucre 5.1-6.4 mm, turbinate, scarious, woolly or glabrate; outer involucral bracts ovate-lanceolate, subacute, the inner oblong, obtuse; florets about 10; scales amongst the florets numerous.	Capitula 5.1 mm long, numerous (10-40), closely-packed, in terminal globose corymbs, turbinate (obconic), on tomentose, very short (tomentose) pedicels; involucral scales few, imbricating, scarious, woolly, inner with white spreading tips; florets 8-10; scales amongst the florets numerous. (detailed description in Hooker 1847)	

The taxonomic status based on morphology of some '*Cassinia*' species has been uncertain and subject to changes (Section 1.1, and Appendix 1 Table 1). Local variation within New Zealand '*Cassinia*' is considerable (Webb 1988), and previously-used morphological characters vary in their taxonomic value. Characters such as leaf shape and size, and number of receptacle scales have been found to vary continuously within and between '*C. leptophylla*' populations (Webb 1988).

Since the publication of the most recent flora treating New Zealand '*Cassinia*', there have been no studies of the morphology of the entire group, although observations on some of the "species" occur in publications on the Gnaphalieae (e.g., Ward 1993, Breitwieser & Sampson 1997a,b).

Numerical taxonomy

Numerical taxonomy as described by Sneath and Sokal (1973) calculates overall (phenetic) similarity among the units of study (termed operational taxonomic units, OTUs) and uses this to construct a system of classification. It differs from earlier phenetic methods in being more objective and repeatable.

Numerical taxonomy is composed of two distinct phases. The first can be defined as all the steps leading up to the production of a data matrix, i.e. OTU and character selection, definition of character states, and data collection. In the second phase the data matrix is analysed to reveal patterns among the OTUs. Some measure of similarity or dissimilarity is computed for each pair of OTUs. This is usually either a generalised distance measure, based on a generalisation of Euclidean distance, or some form of correlation coefficient. In the final computation step, some method of clustering is applied such that those OTUs that are most similar overall are grouped together. This usually results in a phenogram which may be converted into a classification by selecting a cut-off level for each taxonomic rank, and identifying the clusters that are distinct at the cut-off level as the taxa of that rank. Numerical taxonomy groups OTUs on the basis of their overall similarity (or dissimilarity), so that the more features two taxa share, the more likely they will be put in the same group.

Characters in numerical taxonomy

The selection of characters for use in a numerical taxonomic study is, as in any other taxonomic study, of greatest importance, since these characters form the basis of the classification (Sneath & Sokal 1973). A taxonomic or unit character (Sneath & Sokal 1973: 72-75) has to fulfil certain criteria: characters are required which both vary within the total sample (since invariant characters do not provide any information about groupings within the sample) and do not vary within the OTUs, or at least do not vary as much within as between OTUs. Logically correlated characters are to be avoided since they present the same information more than once, which is a form of unequal weighting. Characters that are not logically correlated, but are highly correlated empirically, should be included unless they are known to be caused by a single factor.

Practical criteria are also important for the choice of a character. Characters that are cost/benefit effective should be chosen. Characters that are very time-consuming to explore may be rejected in favour of more quickly accessible data. Fresh material may not be available so that characters that are not retained in preserved or dried material may be rejected for this reason.

Once the characters have been selected, they are treated as of equal significance *a priori*, that is, before classification has begun. Unit characters receive unit weight. The requisite minimum number of characters for a numerical taxonomic study is not known. More characters are assumed to give a more stable pattern of relationships (preferably more than 60 in any given analysis according to Sneath and Sokal, 1973: 106).

Aim and outline

My aim is to quantify the morphological variation present in *Ozothamnus leptophyllus*. I will determine groups within *O. leptophyllus*, based on overall similarity, using analyses of unweighted morphological characters.

Firstly the physical resources (herbarium, garden, field, laboratory) and the selection of the OTUs and characters will be described, followed by a description of the nature and distribution of the morphological characters and an explanation of the numerical technique. The results of the numerical analyses will be given and discussed.

2.2 Material and Methods

2.2.1 Assembling and selecting the plants

Herbarium specimens

Herbarium specimens provide the opportunity for assessment of morphological variation within the New Zealand *Ozothamnus* complex throughout its geographic range. Examination of labels of herbarium specimens can also reveal information on ecology and reproductive period. Herbarium specimens were examined from following herbaria: Allan Herbarium, Landcare Research, Lincoln, New Zealand, (CHR); Department of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand, (CANU); Museum of New Zealand Te Papa Tongarewa, Wellington, (WELT); Auckland War Memorial Museum, Auckland, New Zealand (AK); Otago Regional Herbarium, Botany Department, University of Otago, Dunedin, New Zealand (OTA). Type specimens of all described taxa of *Ozothamnus* in New Zealand were requested and examined (Appendix 1 Table 1). A working herbarium was established holding voucher material of all specimens collected for this study. Standard herbarium practice was adopted according to Benson (1962) and Davis and Heywood (1963).

Field observation and collecting

Plant material was collected in the field from healthy plants in 1998, 1999 and 2000. Specimens were collected from a broad selection of sites (Fig. 2.1, Appendix 3) in order to sample as much of the diversity as possible. Sites were chosen subjectively based on prior knowledge of variation, distribution and accessibility obtained from herbarium and literature studies.

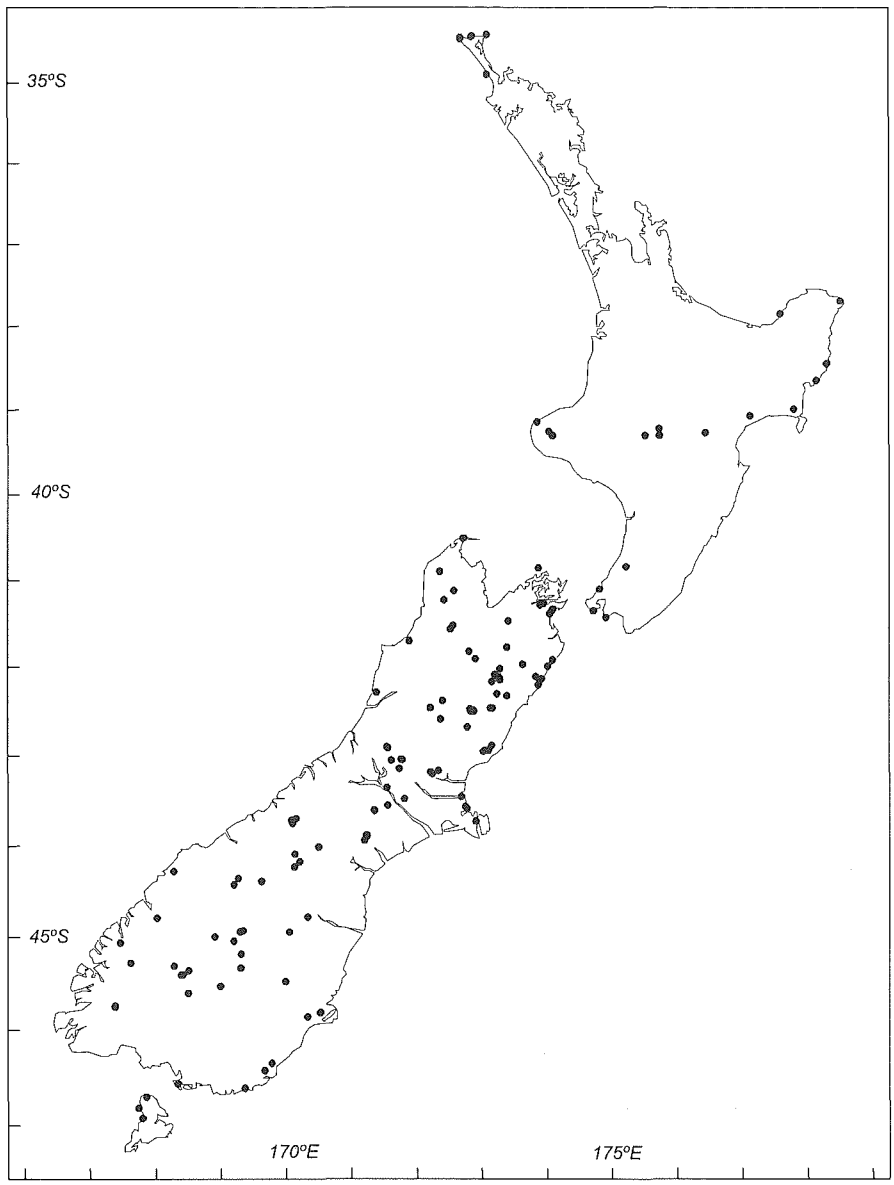


Fig. 2.1 Field collection sites of *Ozothamnus leptophyllus*.

Vegetative and reproductive plant parts were fixed in formalin acetic alcohol (FAA): (16 parts 70% ethanol, 1 part glacial acetic acid, 1 part formalin), pressed as voucher specimens, and cultivated from cuttings. Data were recorded in the field for each specimen collected, including information on habit, habitat, associated flora and phenology. The Cass population was chosen to represent homogeneous populations and sampled by making a diagonal transect across the population and collecting samples every 3 m for a total of 36 m. One to three specimens were collected from all other populations judged to be

homogeneous. A range of specimens representative of the variation present was collected from each polymorphic population. Collection data for all field samples are given in Appendix 3.

Experimental cultivation

The objectives of experimental cultivation were to establish the level of environmentally induced variation and to provide fresh plants throughout the year for the morphological and molecular studies. Of special interest was the generation of flowering material in cultivation to supplement vegetative material collected in the field.

Samples representing the range of variation found within *Ozothamnus leptophyllus* throughout New Zealand were propagated by cuttings and grown under uniform conditions at the University of Canterbury. Propagation by cuttings was performed as described by Bryant (1992) and Follett and Foggo (1981). Five to ten tip cuttings (3-6 cm of vegetative shoots) per specimen were taken from fresh field material. The cuttings were wounded by removing the bottom leaves and dipped into 'Seradix' No. 2 root-forming hormone powder for medium hardwood (3 g/kg beta-Indolylbutyric Acid in form of dust (Rhône-Poulenc)). They were placed into plastic propagation trays with Perlite. The trays were watered by an automatic mist system operated by an electronic 'leaf' in a propagation unit. After the cuttings developed roots (4-12 weeks) they were transferred into plastic pots with potting mix (60% bark, 20% peat, 10% sand, 10% sterilised soil) and cultivated in an unheated glasshouse. The pots were watered daily. As soon as the plants were big enough (15-50 cm high), after approximately 10-20 weeks, they were re-potted, and one specimen from each genotype was planted outside in open ground. Rows of Weedtex cloth, 90 cm wide, were laid down on the local silt loam at ~50 cm distance from each other. Plants were planted in slits cut into the cloth at about 50 cm apart. Bark was put down between the rows. The experimental garden is shown in Figures 2.2 and 2.3. For the garden plan see Appendix 4. The planting was carried out throughout January-May 1999 and September 1999-March 2000, depending on availability of healthy and strong plant material. During the summer months the plants were watered as necessary. Table 2.2 shows a summary of the weather conditions recorded for the Christchurch Botanic Gardens, located c. 3 km from the University of Canterbury.

Table 2.2 Summary of the records of temperature, rainfall and sunshine measured at Christchurch Gardens (New Zealand Climate Digest January 1999 - August 2000, National Institute of Water & Atmospheric Research Ltd).

Month	Air Temperature								Rainfall				
	Means of		Means of	Differ-ence	Absolute Max and Min				Total Fall	No. of	Differ-ence	Max	
	A Max	B Min	A and B	From Normal	Max	Date	Min	Date		Rain Days	From Normal	Fall	Date
	°C	°C	°C	°C	°C		°C		mm		mm	mm	
January 1999	22.6	13.7	18.2	+1.2	28.5	12	10.5	9	32	9	-21	19	31
February 1999	22.7	12.8	17.8	+0.8	30.7	17	5.2	2	35	4	-5	30	26
March 1999	22.3	12.4	17.4	+2.0	31.6	29	6.8	3	49	9	-12	17	23
April 1999	17.1	8.1	12.6	+0.0	24.0	2	0.8	18	60	11	-8	14	28
May 1999	17.1	5.7	11.4	+2.2	25.8	16	0.2	4	41	6	-33	29	5
June 1999	12.9	2.6	7.8	+1.3	22.3	1	-2.0	29	55	12	-4	21	13
July 1999	10.9	2.8	6.9	+0.8	17.3	2	-2.4	10	174	16	+102	51	17
August 1999	12.6	2.6	7.6	+0.1	20.1	24	-3.8	1	57	12	-1	12	5
September 1999	15.5	5.1	10.3	+0.4	23.8	30	0.5	10	34	11	-8	17	20
October 1999	18.1	8.5	13.3	+1.2	24.5	27	2.5	14	44	12	-1	9	5
November 1999	18.4	9.5	14.0	-0.1	27.0	7	3.3	13	60	14	+14	19	4
December 1999	19.8	9.4	14.6	-1.2	26.7	12	3.7	26	33	4	-17	23	13
January 2000	20.4	11.4	15.9	-1.1	30.2	21	5.6	27	60	13	+7	23	3
February 2000	21.8	11.7	16.8	-0.2	32.0	24	6.5	3	24	7	-16	12	17
March 2000	20.1	10.2	15.2	-0.2	30.1	19	1.0	23	56	3	-5	37	13
April 2000	17.3	8	12.7	+0.1	26.0	12	2.5	26	58	15	-10	12	18
May 2000	15.0	5.6	10.3	+1.1	22.8	1	-0.3	17	54	6	-20	28	7
June 2000	13.2	3.4	8.3	+1.8	20.3	25	-1.2	9	49	8	-10	27	4
July 2000	12.3	4.6	8.5	+2.4	16.3	28	0.0	24	19	13	-53	4	19
August 2000	12.9	2.5	7.7	+0.2	19.1	10	-5.2	3	105	12	+47	44	18

Fig. 2.2 Experimental garden, February 1999, after the first plantings.

Fig. 2.3 Experimental garden, November 1999, plants well established, bark between the rows.

OTU selection

After the first survey of herbarium specimens, preliminary groups were formed based on the overall appearance of the plants and their provenance. This provided a method by which to order and catalogue all newly collected material. The groups so-formed were used as a guideline for selecting of specimens for study. A total of 177 populations from throughout the country were collected and sorted into groups using this method. Ninety populations from the original 177 populations were chosen for detailed macromorphological examinations, covering the distribution and observed variation of *Ozothamnus leptophyllus* throughout New Zealand. From some of these populations more than one representative was included in further studies, as well as, in some cases, cultivated and field material of the same plant. This resulted in a total OTU number of 192 (Data Set 1). A three-step sequential reduction procedure based on overall similarities using numerical analysis (Section 2.2.3.5) was performed. The objective was to reduce the number of OTUs in order to more or less equate with the 23 OTUs used for the other areas of investigation, microcharacters and molecular analysis (Chapter 3).

In a first step the initial number of 192 OTUs (Data Set 1) was reduced down to 112 (Data Set 2). This was done by excluding some of the often incomplete field samples of plants which were also represented by cultivated material, and representatives of homogeneous populations of which more than one representative was originally included. In a further reduction step 29 OTUs, primarily from polymorphic populations and identified as putative hybrids, were removed. The resulting Data Set 3 was further reduced by removing OTUs morphologically similar to those that were also included in microcharacter and molecular studies. Based on overall similarity levels, the OTU number could not be reduced below 33. The 'reduced' data set, Data Set 4, therefore contained 10 additional OTUs that were not included in the other areas of investigation.

2.2.2 Data collection

Selection of characters and character states

Characters were selected following an initial survey of taxa, including herbarium specimens and fresh material, representing all major groups and covering as much known variation as possible. An assessment was made of the conservative nature of characters, in the sense of intuitively assessing their variation within and between individuals and populations. Characters were required which both vary within the total sample and do not vary within the OTUs, or at least do not vary as much within as between OTUs. Character lists created by Kalin (1969a, b) for the taxonomic study of the geographic variation and hybridisation in populations of two '*Cassinia* species' were taken into account during the choice of characters. Similarly the main diagnostic characters used in the Floras (Hooker 1853, 1864; Kirk 1899; Cheeseman 1906, 1925; Allan 1961) were considered. However, reliance was not placed entirely on characters described in the literature since these were likely to be biased in favour of prior classification. Unit characters and character states (conditions or expressions of characters) chosen for this study are given in Table 2.5. The botanical terms for characters and character states follow Stearn (1992).

Dissection and assessing of vegetative and floral material

Dissections were carried out under a Wild stereoscopic light microscope (M3C, Wild Heerbrugg Switzerland) with ring illuminator. One of the 10x eyepieces was furnished with a graticule in mount, suitable for precise measurements. Click-stops for the total magnifications 6.4x, 10x, 16x, 25x, 40x were available. The dissecting microscope was fitted with a drawing tube (Type 308700, Wild Heerbrugg Switzerland).

Vegetative and floral material examined came from fresh field material, fresh cultivated material, and dry pressed specimens earlier preserved as herbarium vouchers.

Most vegetative characters were measured and assessed from the current year's growth. The growth since the last period of winter rest is indicated in most of the *Ozothamnus* specimens examined by the small size of the leaves that are formed close to the resting period, a fact which was observed previously by Wardle (1963). Measurements of leaf characters were made on 10 mature, healthy leaves on several branchlets of the

same individual. Smaller autumn leaves were avoided.

Before measuring floral parts of dry specimens the whole panicle was rehydrated and softened in hot water to which a drop of detergent had been added. Measurements on capitula were made during or after anthesis. Measurements on florets were carried out past anthesis to avoid variation in size of the corolla, style and stamen tube (Wilton 1997).

Leaf and exudate colours (branchlet exudate colour, colours of ab- and adaxial surfaces of young and old leaves, exudate colours of ab- and adaxial surfaces of young and old leaves) were compared with the Nickerson Color Fan, distributed by the American Horticulture Society, and published by Munsell Color Co. Inc. in 1957. Old leaves were defined as those attached to woody stems, while young leaves were attached to young soft stems.

The colour character states were assessed as shown in Appendix 5 Tables 1-6. Eighteen colour characters (Table 2.5) were initially included in the phenetic study. However, this overweighted the colour characters. Many of these characters were highly correlated and logically interrelated (e.g. colour characters from old and young leaves are both determined by the exudate density and colour). Therefore a bivariate correlation matrix was created in S-Plus 4.5 (r2) (Statistical Sciences 1998) using Pearson Correlation Coefficient (Appendix 6) to analyse the correlation of all colour characters. With the correlation coefficient ≥ 0.66 for a character pair, the association was strong enough to choose just one of these characters for the final analysis. After correlation analysis the 18 colour characters were reduced to eight by excluding the branchlet exudate colour (Characters 7 and 8) and the colour characters of the old leaves (Characters 12, 14, 16, 18, 39, 40, 42, 43).

Microcharacters

Characters from leaf sections, tomentum, achenes, pappus and anthers were selected (Table 2.5) and examined using an Olympus CH compound microscope, which was fitted with a drawing tube (Model BH2-DA, Olympus). Microcharacters were studied in fresh cultivated material, where this was available. However, it was sometimes necessary to study herbarium specimens or material in FAA. Fresh material was dissected and/or sectioned without any preliminary preparation; herbarium material was rehydrated in hot water to

which a drop of detergent had been added. Leaf sections were cut by hand with a razor blade and mounted in water. Measurements were made using a graduated eyepiece. Microcharacters were assessed for only a small number of OTUs (Table 2.3), representing the 10 different groups that had been formed after the first survey (Section 2.3.1). All measurements and observations were recorded in a Microsoft Excel data matrix of 23 OTUs and 15 characters.

Pollen was examined from 20 individuals (Table 2.3), representing the 10 different groups that had been formed after the first survey (Section 2.3.1). All samples were taken from cultivation, except for one that was taken from a herbarium specimen ('*Cassinia amoena*': CHR355755). Florets in which the corolla lobes were unopened or were just opening were dissected and the pollen mounted on an aluminium stub covered with double-sided carbon conductive adhesive tape. The pollen was then coated with gold using a Polaron E5000 splutter coater. All specimens were viewed using a Leica S440 Scanning Microscope fitted with an Oxford Energy Dispersion Spectra Analysis unit. Between 7 and 17 pollen grains were photographed per OTU. The pollen images were visually assessed and grouped according to qualitative descriptive characters. Pollen characters were not included in numerical analyses.

Table 2.3 OTUs included in micromorphological survey ((1) not included in pollen analysis, (2) only pollen has been assessed).

Groups	OTUs
'Fulvida Canterbury Coast.'	7C/fc, 8G/fc
'Fulvida Central Otago/Inland Canterbury'	43A/fc, 34A/fc, 77A/ff(1)
'Leptophylla'	58B/fc, 70A/fc, 139B/fc
'Vauvilliersii West Coast'	13A/fc, 9A/fc(1)
'Vauvilliersii S-Otago/Southland/Fiordland'	17A/fc, 118B/fc, 16A/fc(2)
'Vauvilliersii var. pallida/albida/canescens'	33B/fc, 65A/fc,
'Vauvilliersii Canterbury/Otago/N-Southland'	15A/fc, 4X/fc, 64A/fc, 35A/fc(1), 11A/fc(1)
'Vauvilliersii N-Island'	112A/fc, 148A/d(1)
'Retorta'	105A/fc, 106A/fc
'Amoena'	CHR 355755(2), 158/fc(1)

2.2.3 Numerical analyses

Following selection and measurement of characters and character states, analysis was required to measure overall similarity between each pair of OTUs, and to detect possible groups and subgroups among the OTUs. Resemblance among OTUs was estimated by calculating a coefficient of similarity between each pair of OTUs.

2.2.3.1 Similarity coefficient

All of the similarity matrices for the phenetic analyses were generated using Gower's general coefficient of similarity (Gower 1971). Gower's coefficient is a combination of three different similarity coefficients. One of these is Jaccard's association coefficient (S_J). Used with binary dichotomous characters, Jaccard's coefficient does not score shared absence or negative state of a character as a similarity or match. If OTUs share a positive character state for a binary character, then the similarity is calculated as 1. For qualitative discrete non-ordered characters, the simple matching coefficient (S_{SM}) is used. This simple matching coefficient scores both shared positive character states and shared negative characters as a similarity or match. Alternative dichotomous characters are scored as a match if OTUs being compared are identical for the character state, whether this is presence or absence. Gower employs the simple matching coefficient for this character type also.

Jaccard's coefficient is defined as:

$$S_J = Nsp / (Nsp + Nu)$$

The simple matching coefficient is defined as:

$$S_{SM} = (Nsp + Nsn) / (Nsp + Nsn + Nu)$$

Where Nsp is the number of states whose presence or positive state is shared by two OTUs, Nsn is the number of shared negative states in the two OTUs being compared and Nu is the number of unshared states (the number of characters in which one OTU is positive/present and the other negative/absent). For quantitative and ordered qualitative characters, the majority of characters used in the morphology part of this study, Gower applies the following coefficient:

$$S_{ijk} = 1 - (| X_{ik} - X_{jk} | / R_k)$$

Where X_{ik} is the score of OTU i for character k, X_{jk} is the score of OTU j for character k and R_k is the range of character k.

The calculation of the Gower coefficient for all pairs of OTUs from a rectangular data (OTU x character) matrix produces a symmetrical similarity (OTU x OTU) matrix in which a measure of similarity between every pair of OTUs is expressed. The similarity matrices for all sets of OTUs were created using the routine "gower5" developed by Wilton (1999) to run Gower's general coefficient of similarity within S-Plus 4.5 (r2) (Statistical Sciences 1998).

Defining groups of related OTUs based on high similarity coefficients is the next step in the phenetic approach. The two main ways to do this are a) clustering and b) ordination.

2.2.3.2 Clustering

Cluster analysis is a term used to describe a set of numerical techniques in which the main purpose is to cluster the objects of study into discrete groups based on the objects' characteristics.

Cluster analysis is used in many scientific disciplines and a wide variety of techniques have been developed to suit different kinds of studies. The most commonly used clustering methods in biology are the sequential, agglomerative, hierarchic, non-overlapping ones (SAHN). The SAHN clustering algorithm proceeds as follows:

1) This similarity matrix is scanned to find the pair of OTUs with the highest similarity (or least distance). These are joined at the level of their similarity.

2) The cluster formed by these two OTUs can now be considered a single object. The similarity matrix is recalculated so that all the other cases are compared with this new group, rather than the original two OTUs.

3) The modified matrix is then scanned (as in step 1) to find the pair of OTUs or clusters that now have the highest similarity.

Steps 2 and 3 are repeated until all the objects have been combined into a single group.

The result of cluster analysis can be displayed as a dendrogram that shows the most similar OTUs linked most closely together. The level of the lines joining two OTUs or clusters indicates the level of similarity between them. It is important to note that the branching hierarchy and the level of similarity are the only important features of the dendrogram. The order of the OTUs is not significant. The dendrogram can be envisaged as a mobile that allows the individual clusters to rotate around.

There are several types of agglomerative clustering methods commonly in use. These all follow the basic algorithm outlined above, varying only in the manner in which the similarity between clusters is calculated.

Single linkage and complete linkage clustering

With single linkage or nearest neighbour clustering, the distance between one group and another is taken as the distance between their two closest points. This means that an OTU has a similarity to an existing cluster that is equal to its similarity to the closest member within the cluster. Single linkage clustering is not generally popular for taxonomic investigations because it produces dendrograms that are unsuitable for translation into systems of classification. This is because such dendrograms tend to have a poor hierarchical structure, due to chaining. Chaining is a phenomenon in which there is a tendency to repeatedly add new OTUs onto a single cluster rather than making several separate clusters. This gives the dendrogram a staircase-like appearance. Single linkage also tends to cluster over a relatively small range of similarity values. It was used here because it may provide useful information in comparison with other methods.

Complete linkage clustering, or furthest neighbour technique, is the antithesis of the single linkage method. It takes the distance between the two furthest points as being that between the two groups. The clusters produced by this method show usually induced compactness by comparison with the loose, strung-out single linkage clusters.

Single linkage and complete linkage clustering may be simple, but they can also be viewed as distorting the data, since the distances between groups are calculated based on what may be unusual outlying points rather than the properties of the whole cluster.

Average linkage clustering

With these techniques, the distances between groups are represented as an average distance. There are two basic approaches. Firstly, the average method measures the distances between each pair of points in the two clusters and takes the mean of these distances as the distance between the clusters. In the second, the centroid of each group is calculated and the distance between the groups is represented by the distance between the centroids. The centroid itself can be described as the average point of the cluster. It is calculated by taking the mean value of the coordinates on each axis for all the points in the cluster.

There are also two variants that apply to both of these methods; the calculations can be either weighted or unweighted. The unweighted methods give equal weight to each point in each cluster. The weighted methods instead give equal weight to each cluster; if one cluster has fewer points than another, those points in the smaller cluster must be given higher weighting in the calculations to make the two groups equal. In general, the unweighted versions are used unless the data are expected to have some clusters that are much smaller than others (e.g. if some groups have been sampled less than others).

This study will be using as a basis the unweighted pair-group method using arithmetic averages (UPGMA). UPGMA clustering is used extensively in taxonomic studies since it tends to combine a clear hierarchical structure with a reasonably accurate reflection of the phenetic relationships shown in the similarity matrix. The clusters form over an intermediate range. UPGMA generally gives the least amount of distortion of a similarity matrix (Sneath & Sokal 1973). One problem, however, is that outlying OTUs (those which are not similar to any others) may form a pair not because they are most similar to each other, but rather because their similarity to each other is higher than either one's average similarity to any existing cluster.

The weighted pair-group method using arithmetic average (WPGMA) was used to supplement results from UPGMA clustering, since it is less sensitive to cluster size than UPGMA. It was used to uncover any distortions in the UPGMA dendrogram which might be due to the uneven sample size of different groups. The data were expected to have some variation in cluster size.

The routine "phen.ana5" developed by Wilton (1999) to run phenetic analyses in S-Plus 4.5 (r2) (Statistical Sciences 1998) was applied to the similarity matrices in this study.

2.2.3.3 Ordination

The term 'ordination' derives from early attempts to order a group of objects, for example in time or along an environmental gradient. It is the ordering of a set of data points with respect to one or more axes. Alternatively it can be seen as the displaying of a swarm of data points in a two or three-dimensional coordinate frame, so as to make visible the relationships among the points in many-dimensional spaces (Pielou 1984). Sneath and Sokal (1973) described ordination as "placement of t OTUs in an A-space of dimensionality varying from 1 to n or t -1, whichever is less, a summary of the information about relationships implied by the entire suite of characters".

There are numerous ordination techniques that allow a concise and visually easy-to-grasp representation of very complex data sets. The most common ones are those in which the structure of groups is optimised in a simultaneous fashion. Of these common techniques, principal component analysis (PCA) and principal coordinate analysis (PCO) are most often employed.

Principal component analysis (PCA) is one of the best known and earliest ordination methods, first described by Karl Pearson (1901). Mathematically, PCA consists of an eigenanalysis of a covariance or correlation matrix calculated on the original measurement data. Graphically, it can be described as a rotation of a swarm of data points in multidimensional space so that the longest axis (the axis with the greatest variance) is the first PCA axis, the second longest axis perpendicular to the first is the second PCA axis, and so forth. Thus these first few PCA axes represent the greatest amount of variation in the data set and usually contain patterns of significance.

The first step of a PCA is the calculation of the covariance or correlation matrix for the characters. The correlation matrix is used if standardisation is desired; this is useful if the characters have been measured on different scales or are of different orders of magnitude. Otherwise the covariance matrix should be used (Thorpe 1983, Kovach 1998b). An eigenanalysis is then performed on the matrix.

Principal coordinate analysis (PCO), developed by Gower (1966) can be viewed as a more general form of PCA. Whereas in PCA the use of a covariance or correlation matrix is implicit, PCO can use a variety of different measures of distance or similarity. It then performs an eigenanalysis of the matrix, giving eigenvalues and eigenvectors. In general, the distances or similarities are measured between the OTUs directly, rather than the

characters as in PCA, and the eigenvectors represent the scores for the OTUs. It thus gives a direct ordination of the OTUs and is useful in situations where there are more characters than OTUs (PCA is not recommended under this circumstance) (Gower 1966). The main advantage of PCO is that many different kinds of similarity or distance measures can be used. For instance, if you are working with mixed data, with quantitative and characters, Gower's general similarity coefficient can be used to combine these data. These coefficients can then be analysed using PCO, whereas this data matrix could not be analysed by other ordination methods without recoding the data into a single form.

Gower's distance matrices created by the routine "Gower5" of the Phenetic Library for S-Plus 4.5 (r2) (Wilton 1999) were imported into MVSP Plus Version 3.0 (Kovach 1998) where PCO was carried out.

2.2.3.4 Tests of correlation between matrices

Cophenetic correlation coefficient

It is important to check for the validity of clusters, because 1) clustering methods will always carry out their function whether or not there are groups represented by the data, and 2) the dendrogram used to show the results of cluster analysis is a two-dimensional representation of a multi-dimensional structure. Calculation of the cophenetic correlation coefficient (Sokal & Rohlf 1962) is the simplest way to find the degree of fit of a dendrogram to the similarity matrix from which it is derived. It determines the correlation of the original similarities with the "cophenetic values" (linkage levels, the clustering levels calculated from the dendrogram). The cophenetic correlation coefficient was calculated for each dendrogram created during this study using both Pearson Correlation Coefficient and Spearman's Rank Correlation Coefficient.

The Pearson Correlation Coefficient is the most commonly used. It is a measure of the linear association between two variables that have been measured on interval or ratio scales. However, it can be misleadingly small where there is a non-linear relationship between the characters. The Pearson Correlation Coefficient can be also highly influenced by outliers in one or both samples.

Spearman's Rank Correlation Coefficient is a sensible alternative to Pearson Correlation Coefficient when normality is unreasonable or outliers are present. The Spearman Rank Correlation Coefficient is the Pearson Correlation Coefficient computed from the pairs of ranks. It is not sensitive to the presence of outliers in the data.

Cophenetic correlation values were calculated within the routine "phen.ana5" (Wilton 1999).

Mantel test

Often one wishes to test whether one set of relationships among a set of objects is independent of or congruent with another. The Mantel test (Mantel 1967) is a commonly used statistical tool that can be used to determine the significance of a correlation between two matrices (Sokal 1979). The test assumes that the two matrices have been obtained independently - one cannot use it to test two matrices where one has been derived from the other. The test criterion is

$$Z = \sum_{i<j}^{n} X_{ij} Y_{ij}$$

where X_{ij} and Y_{ij} are the off-diagonal elements of the matrices X and Y. If the two matrices show similar relationships, the Z should be large in comparison to what one would expect by chance. Mantel also computes the product-moment correlation, *r* (Rohlf 1963, Sneath & Sokal 1973).

Mantel tests were performed to compare the similarity matrix based on floral data with that based on vegetative data using the matrix comparison routine MXCOMP in NTSYS-pc (Rohlf 1997).

2.2.3.5 Reduction of the OTU number using numerical analyses

Initially 192 OTUs (Data Set 1), representing 90 different populations and including both fresh, cultivated and dry field specimens, were subjected to numerical analysis. The degree of similarity between each pair of OTUs was calculated, based on 80 characters, using Gower's general coefficient of similarity. The similarity values were compiled into a similarity matrix, which was used as a basis for grouping specimens by cluster analysis.

Four methods of clustering were used: unweighted pair-group method using arithmetic averages (UPGMA), weighted pair-group method using arithmetic average (WPGMA), single linkage clustering, and complete linkage clustering (Section 2.2.3.2).

The results were presented in the form of phenograms. The overall cophenetic correlation coefficient was calculated for all four phenograms using both Pearson Correlation Coefficient and the Spearman's Rank Correlation Coefficient to find the degree of fit of each phenogram to the similarity matrix from which it was derived.

Clusters joining at a relatively high level of similarity (> 0.9) in the average linkage phenograms were compared with clusters in the WPGMA, single linkage and complete linkage phenograms. A representative from each cluster of the UPGMA phenogram was selected for a reduced Data Set 2.

Data Set 2, containing only 112 OTUs was subjected to the same numerical analyses as for Data Set 1. The resulting clusters for each algorithm (see above) were compared. Larger clusters, or those of particular significance, were given a code corresponding to the groups defined after the first survey (Section 2.3.1).

To identify putative hybrids and intermediate forms, PCO was performed on Data Set 2. Gower's distance matrix based on Data Set 2, and created by the routine "Gower5" of the Phenetic Library for S-Plus 4.5 (r2) (Wilton 1999), was imported into MVSP Plus Version 3.0 (Kovach 1998) where PCO was carried out. Seven clusters formed in the UPGMA phenogram based on Data Set 2 and 5 polymorphic populations containing putative hybrids were depicted on the PCO plots.

Based on the analyses of Data Set 2 the OTUs were further reduced. OTUs from polymorphic populations were excluded from further analyses and the remainder were selected for a further reduced Data Set 3. This was subjected to the same numerical analyses as for the earlier two data sets, and with the help of the results further reduced by selecting representatives from each cluster.

2.2.3.6 Numerical analyses of the reduced data set

A Data Set 4, containing 33 OTUs and 80 characters was subjected to the following analyses:

Clustering

Four cluster algorithms (the unweighted pair-group method using arithmetic averages (UPGMA), the weighted pair-group method using arithmetic averages (WPGMA), single linkage clustering and complete linkage clustering) were performed on Data Set 4, but only one of the resulting phenograms, the UPGMA phenogram, was used to identify and describe groups and clusters in detail. The UPGMA clusters were then compared with the results of the other three clustering methods and with the result of UPGMA clustering of Data Set 3.

Ordination

Gower's distance matrix based on Data Set 4, and created by the routine "Gower5" of the Phenetic Library for S-Plus 4.5 (r2) (Wilton 1999), was imported into MVSP Plus Version 3.0 (Kovach 1998) where PCO was carried out. Six clusters (V, L, F, R, A, and ALB) formed up to the level of 0.735 in the UPGMA phenogram based on Data Set 4 were depicted on the PCO plots.

Character box-plots

Differences among groups were determined by producing a character breakdown of character distributions according to the UPGMA phenogram based on Data Set 4 and Data Set 6 for the additional microcharacters (Section 2.2.3.7). This was done by using the routine "Phenogram analysis" added in 2001 by Wilton to the Phenetic Library for S-Plus 4.5 (r2) (Wilton 1999) and visualised with box-plots. These displayed the upper extreme (excluding outliers), upper quartile, median, lower quartile, and lower extreme (excluding outliers) for each character box. By default, anything beyond 1.5 times the Inter-Quartile Range is considered an outlier. Whiskers are drawn to the nearest value not beyond a standard span from the quartiles; points beyond whiskers (outliers) are drawn individually. The standard span is 1.5 (Inter-Quartile Range).

2.2.3.7 Numerical analyses of reduced data sets with additional microcharacters and a character subset containing microcharacters only

Data Set 5 was prepared, comprising all the 33 OTUs and the characters (excluding those listed below) from Data Set 4, plus 15 additional microcharacters. Five characters (61 = corolla tube length, 85 = pappus tip thickening, 89 = density of twin hairs, and 90 = density of glandular hairs) were removed from the main character set because they are descriptive versions of some of the microcharacters. Microcharacters were available for only 23 of the 33 OTUs so that the new data matrix contains several 'missing values'. UPGMA cluster analysis was performed on Data Set 5.

The 10 OTUs without microcharacters were then excluded from further analysis. The resulting Data Set 6 with 23 OTUs was subjected to UPGMA analysis. Box-plots for each microcharacter were created to visualise the character distribution within each of the 6 groups given by the UPGMA phenogram based on Data Set 6 at a similarity level of 0.72.

Data Set 7, based on Data Set 6 but with the 15 microcharacters only, was created. A UPGMA cluster analysis was carried out based on this data set with 23 OTUs and the 15 microcharacters.

2.2.3.8 Numerical analysis of character subsets; vegetative or floral characters

Two character subsets (Data Sets 8 and 9) based on Data Set 4 were prepared. Data Set 8 contained 33 OTUs and the vegetative characters (characters 1-47). Data Set 9 was composed of 31 OTUs and the floral characters (characters 48-90). The OTUs 158/fc and 159A/d were excluded from Data Set 9 because no floral characters were available for these specimens. Both subsets were analysed using Gower's general coefficient of similarity and UPGMA clustering.

Similarity matrices for vegetative and floral characters (Data Sets 8 and 9) were transferred to NTSYS-pc (Rohlf 1997) for analysis using the Mantel test (Mantel 1967) with 1000 iterations to test for significant correlation between the data sets and to compute the product-moment correlation, *r*. Missing data contained in matrix 9 were identified using "999" in NTSYS. Data Sets 8 and 9 were then pooled for matrix comparison.

2.3 Results

2.3.1 Grouping on the basis of the overall appearance of the plants

Classification using the overall appearance of the field-collected plants resulted in 10 preliminary groups (Table 2.4), which can themselves be grouped according to leaf size. Members of each group are named according to previously used taxa names and/or according to their geographical distribution. The groups are described as follows:

A) SMALL-LEAVED SPECIMENS: This group includes coastal and lowland populations with slender branchlets and small, narrow leaves (1.5-3 x 1-1.5 mm).

'Fulvida Canterbury Coast': This group contains shrubs up to 2 m tall with an untidy appearance. The branchlets are long and slender, more or less fastigiate, sometimes descending or deflexed. The new branchlets below the terminal inflorescence are often well developed while the flowers are still in bud. The leaves are small and densely set. Coloured exudates on leaves and branchlets give the plant a yellow appearance. The panicles are large, and more or less spreading. The capitula are small and very numerous per panicle. Representatives of this group are coastal or lowland specimens from the Canterbury and Marlborough Coast.

'Fulvida Central Otago/Inland Canterbury': This group includes only eight small populations, which are very similar to the 'Fulvida Canterbury Coast' group. The panicles are slightly smaller and the exudates, responsible for the colour of leaves and branchlets, are dark yellow. The main reason for establishing this as a new group is that these populations occur more inland, far away from coastal habitats.

'Leptophylla': The general appearance of the specimens in this group is not much different from that of the representatives of the two 'Fulvida' groups above. The main difference is the reduction or absence of yellow exudates. The white tomentum gives these plants a greyish-white appearance. The 'Leptophylla' group includes only coastal populations from the North Island and the North of the South Island.

'Retorta': Representatives of this group resemble specimens belonging to the 'Leptophylla' group in their small densely set leaves with little or no coloured exudates. The shrubs are smaller and the new branchlets below the terminal inflorescence do not start to elongate before the flowers have fruited and died. The shoots are shorter and stouter.

The panicles consist of only a few very large capitula. The specimens grouped together in the 'Retorta' group are entirely coastal and were found only in the North Auckland District.

B) LARGE-LEAVED SPECIMENS: This group includes plants with stout branchlets and larger leaves (4-7 × 1.5-3 mm).

'Vauvilliersii West Coast': The representatives of this group are small shrubs with several main branches of which some, mostly the inner ones, are upright and the outer ones are decumbent or prostrate. The leaves are quite large, densely set, and dark green with only a few yellow exudates. The panicles are small. The populations in this group inhabit wetlands of high mountains on the west side of the Main Divide.

'Vauvilliersii South-Otago/Southland/Fiordland': This group contains tall upright rounded 'tidy' shrubs (up to 2 m tall) with large, dark green shiny leaves. Yellow exudates are present on the abaxial surface of the leaves and on the branchlets. The panicles are small and dense and the outer involucral bracts quite frequently have red tips. Populations mostly from lowland shrubland and grassland in South Otago, Southland, and Stewart Island group fall into this group.

'Vauvilliersii var. pallida/albida/canescens': The representatives of this group are upright shrubs with large leaves. The stout branchlets and leaves are clad in dense tomentum. The adaxial surface of the leaves retains most of the white tomentum when mature. The plants lack yellow exudates but branchlets and leaves are very glutinous throughout the season. Plants formerly classified as *Cassinia vauvilliersii* var. *pallida*, *C. vauvilliersii* var. *albida* and *C. vauvilliersii* var. *canescens* fall into this group, which occurs in the Kaikoura Ranges and montane and subalpine shrubland and grassland along river valleys in Marlborough.

'Vauvilliersii North Island': This is an "artificial" group created for the large-leaved specimens from higher altitudes in the North Island to ensure that the limited number of collections in this study are included in detailed molecular and morphological studies.

'Vauvilliersii Canterbury/Otago/N-Southland': This largest group in this study contains populations from mountain ranges in Canterbury, Otago and North Southland. Yellow exudates give the tidy, more or less rounded bushes a yellow appearance. The leaves are slightly smaller and the branchlets are less stout than in the representatives of the

above 'Vauvilliersii' groups.

'Amoena': The representatives of this group are known only from cliffs from Kerr Point to North Cape. They are small shrubs (hardly up to 1 m tall). The stout branchlets and the abaxial surface of the leaves are densely white-tomentose. The tomentum extends to the adaxial surface of the leaves.

C) POLYMORPHIC POPULATIONS: In some populations, mainly in Marlborough, plants of distinctly different appearance can be found growing next to each other. Small leaved forms from the 'Fulvida Canterbury Coast' and the 'Leptophylla' groups can occur in one and the same coastal population. Polymorphic populations from higher altitudes contain large leaved specimens (mainly from the 'Vauvilliersii var. pallida/albida/canescens' group) together with small leaved specimens and a wide range of intermediate forms.

Table 2.4 Field population samples grouped on the basis of overall appearance of the plants.

Groups	Population Number																					
Fulvida Cant. Coast. (FCC)	5	6	7	8	10	12	18	19	20	21	22	31	52	56	57	77	100	164				
Fulvida Central Otago/Inland Canterbury (FOC)	34	42	43	45	46	47	73	145														
Leptophylla (L)	58	70	71	72	80	81	94	96	101	121	122	123	137	138	139	140	154	157	166	167	168	172
Vauvilliersii West Coast (VWC)	9	13	74	75	76	91	119	150	153													
Vauvilliersii S-Otago/ Southl./ Fiordland (VOSF)	16	17	97	98	111	114	115	116	117	118	120	152	144	162	170	175	176					
Vauvilliersii var. pallida/ albida/canescens (VPAC)	11	33	65	66	67	68	78	79	84	99	109	110	126	128	129	159	160	173				
Vauvilliersii Cant./Otago/N-Southland (VCOS)	4	14	15	35	36	37	38	39	40	41	44	48	49	50	51	53	59	60	61	62	63	
	64	69	82	83	85	86	87	88	89	90	92	95	102	103	113	124	125	127	130	131	132	133
	134	135	136	151	155	156	161	163	177													
Vauvilliersii N-Island (VN)	112	146	147	148	165	169	171															
Retorta (R)	104	105	106	107	108																	
Amoena (A)	158	CHR *	CHR *																			
Polymorphic populations (PP)	1	2	3	23	25	26	27	28	29	30	32	54	55	131	141	142	143	149	174			

DNA Extractions population included in morphological analysis * Herbarium specimens CHR 389520 and CHR 355755

2.3.2. Morphological characters

The following section describes the morphological characters used in this study. Character states and classes are shown in Table 2.5. The statistics of absolute values for the quantitative multi-state characters are given in Appendix 7.

Table 2.5 Characters, character states, and character classes. (☐ Characters excluded from analyses.)

	Character	Character states	Character classes
1	habit	1 = plant upright, slender with long shoots; 1.5 = habit between 1 and 2; 2 = plant upright round, compact with stout shoots; 2.5 = habit between 2 and 3; 3 = plant with upright and prostrate shoots	*Ordered qualitative multi-state character*
2	plant height	1 = not more than 80 cm; 2 = between 80 and 150 cm, 3 = over 150 cm	*Ordered qualitative multi-state character*
3	branchlet angle	average of 10 measurements of new season growth (degrees)	*Quantitative multi-state character*
4	branchlet diameter	average of 10 measurements of new season growth (mm)	*Quantitative multi-state character*
5	branchlet indumentum thickness	1 = sparse, 2 = moderate, 3 = thick, 4 = very thick, 5 = extremely thick	*Ordered qualitative multi-state character*
6	branchlet exudate density	0 = absent; 1 = sparse, 2 = moderate, 3 = dense, 4 = very dense, 5 = extremely dense	*Ordered qualitative multi-state character*
7	branchlet exudate colour gradient from green to yellow	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 1)	*Ordered qualitative multi-state character*
8	branchlet exudate colour depth	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 2)	*Ordered qualitative multi-state character*
9	branchlets glutinous	0 = not, 1 = slightly, 2 = strongly, 3 = very strongly, 4 = extremely	*Ordered qualitative multi-state character*
10	branchlet spacing	number of branchlets per 5 cm of new season growth	*Quantitative multi-state character*
11	colour (gradient from green to yellow) of adaxial surface of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 5)	*Ordered qualitative multi-state character*
12	colour (gradient from green to yellow) of adaxial surface of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), character states see Appendix 5 table 5)	*Ordered qualitative multi-state character*
13	colour (depth) of adaxial surface of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 6)	*Ordered qualitative multi-state character*
14	colour (depth) of adaxial surface of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 6)	*Ordered qualitative multi-state character*
15	colour (gradient from green to yellow) of abaxial surface of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 3)	*Ordered qualitative multi-state character*
16	colour (gradient from green to yellow) of abaxial surface of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 3)	*Ordered qualitative multi-state character*
17	colour (depth) of abaxial surface of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 4)	*Ordered qualitative multi-state character*

Table 2.5 continued

	Character	Character states	Character classes
18	colour (depth) of abaxial surface of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 4)	*Ordered qualitative multi-state character*
19	lamina orientation (angle branchlet and lamina)	average of 10 measurements of new season growth	*Quantitative multi-state character*
20	main leaf form (shape of majority (80%) of mature leaves)	1 = oblong, 2 = elliptic, 3 = obovate	*Ordered qualitative multi-state character*
21	secondary leaf form (form of minority (20%) of mature leaves)	1 = oblong, 2 = elliptic, 3 = obovate	*Ordered qualitative multi-state character*
22	main apex	0 = rounded; 1 = obtuse; 2 = acute; 3 = retuse	*Unordered qualitative multi-state character*
23	secondary apex	0 = rounded; 1 = obtuse; 2 = acute; 3 = retuse	*Unordered qualitative multi-state character*
24	length of lamina	average of 10 measurements (mm)	*Quantitative multi-state character*
25	width of lamina	average of 10 measurements (mm)	*Quantitative multi-state character*
26	ratio (length/width)	average	*Quantitative multi-state character*
27	margin recurved	1 = slightly, 2 = moderate, 3 = strongly, 4 = very strongly	*Ordered qualitative multi-state character*
28	midrib evident (protruding)	1 = slightly, 2 = moderate, 3 = strongly, 4 = very strongly	*Ordered qualitative multi-state character*
29	density of trichomes on adaxial surface of lamina of young leaf	0 = absent, 1 = sparse, 2 = moderate, 3 = dense, 4 = very dense	*Ordered qualitative multi-state character*
30	indumentum thickness of abaxial surface of lamina of young leaf	1 = sparse, 2 = moderate, 3 = thick, 4 = very thick, 5 = extremely thick	*Ordered qualitative multi-state character*
31	density of trichomes on adaxial surface of lamina of old leaf	0 = absent, 1 = sparse, 2 = moderate, 3 = dense, 4 = very dense	*Ordered qualitative multi-state character*
32	indumentum thickness of abaxial surface of lamina of old leaf	1 = sparse, 2 = moderate, 3 = thick, 4 = very thick, 5 = extremely thick	*Ordered qualitative multi-state character*
33	exudate colour (gradient green to yellow) on adaxial surface of lamina of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 1)	*Ordered qualitative multi-state character*
34	exudate colour (depth) on adaxial surface of lamina of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 2)	*Ordered qualitative multi-state character*
35	exudate density on adaxial surface of lamina of young leaf	0 = absent, 1 = sparse, 2 = moderate, 3 = dense, 4 = very dense, 5 = extremely dense	*Ordered qualitative multi-state character*
36	exudate colour (gradient green to yellow) on abaxial surface of lamina of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 1)	*Ordered qualitative multi-state character*
37	exudate colour (depth) on abaxial surface of lamina of young leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 2)	*Ordered qualitative multi-state character*
38	exudate density on abaxial surface of lamina of young leaf	0 = absent, 1 = sparse, 2 = moderate, 3 = dense, 4 = very dense, 5 = extremely dense	*Ordered qualitative multi-state character*
39	exudate colour (gradient green to yellow) on adaxial surface of lamina of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 1)	*Ordered qualitative multi-state character*
40	exudate colour (depth) on adaxial surface of lamina of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 2)	*Ordered qualitative multi-state character*
41	exudate density on adaxial surface of lamina of old leaf	0 = absent, 1 = sparse, 2 = moderate, 3 = dense, 4 = very dense, 5 = extremely dense	*Ordered qualitative multi-state character*

Table 2.5 continued

	Character	Character states	Character classes
42	exudate colour (gradient green to yellow) on abaxial surface of lamina of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 1)	*Ordered qualitative multi-state character*
43	exudate colour (depth) on abaxial surface of lamina of old leaf	Nickerson Color Fan distributed by American Horticulture Society (1957), (character states see Appendix 5 table 2)	*Ordered qualitative multi-state character*
44	exudate density on abaxial surface of lamina of old leaf	0 = absent, 1 = sparse, 2 = moderate, 3 = dense, 4 = very dense, 5 = extremely dense	*Ordered qualitative multi-state character*
45	plant glutinous	0 = not, 1 = slightly, 2 = strongly, 3 = very strongly	*Ordered qualitative multi-state character*
46	plant remains glutinous throughout the year	0 = no; 1 = yes	*Two-state (binary) character* Alternative character
47	leaf spacing	number of leaves per cm (average of 10 measurements)	*Quantitative multi-state character*
48	panicle compactness	1 = very spreading, 2 = spreading, 3 = dense, 4 = very dense	*Ordered qualitative multi-state character*
49	number of capitula per panicle	average of 10	*Quantitative multi-state character*
50	panicle diameter	average of 10 measurements (mm)	*Quantitative multi-state character*
51	panicle diameter / (number of capitula per panicle x involucre width)	average	*Quantitative multi-state character*
52	panicle structure	number of peduncle levels	*Quantitative multi-state character*
53	pedicel length	average of 10 measurements (mm)	*Quantitative multi-state character*
54	capitulum top width (diameter)	average of 10 measurements (mm)	*Quantitative multi-state character*
55	capitulum length	average of 10 measurements (mm)	*Quantitative multi-state character*
56	involucre length	average of 10 measurements (mm)	*Quantitative multi-state character*
57	capitulum body width	average of 10 measurements (mm)	*Quantitative multi-state character*
58	ratio of capitulum body width to involucre length	average	*Quantitative multi-state character*
59	ratio of capitulum body width to top width	average	*Quantitative multi-state character*
60	floret number per capitulum	average of 10	*Quantitative multi-state character*
61	corolla tube length	average of 10 measurements (mm)	*Quantitative multi-state character*
62	corolla lobe length	average of 10 measurements (mm)	*Quantitative multi-state character*
63	corolla lobe width	average of 10 measurements (mm)	*Quantitative multi-state character*
64	corolla max. diameter	average of 10 measurements (mm)	*Quantitative multi-state character*
65	style length (without stigmatic lobes)	average of 10 measurements (mm)	*Quantitative multi-state character*
66	stigmatic lobe length	average of 10 measurements (mm)	*Quantitative multi-state character*
67	outer involucral bract shape	1 = narrow-ovate, 1.5 = between 1 and 2; 2 = ovate, 2.5 = between 2 and 3; 3 = wide-ovate	*Ordered qualitative multi-state character*
68	outer involucral bract tip (apex) colour	0 = no, 1 = tawny to transparent; 1.5 = 1 with white edges; 2 = white; 2.5 = 1 or 2 slightly red; 3 = red/pink with tawny edges, 4 = yellow	*Unordered qualitative multi-state character*
69	outer involucral bract tip (apex) spreading	0 = not, 1.5 = between 0 and 1; 1 = slightly	*Ordered qualitative multi-state character*
70	density of trichomes on abaxial surface of outer involucral bracts	1 = sparse, 2 = moderate, 3 = dense	*Ordered qualitative multi-state character*
71	number of outer involucral bracts	average of 10	*Quantitative multi-state character*
72	inner involucral bract length	average of 10 measurements (mm)	*Quantitative multi-state character*
73	inner involucral bract width	average of 10 measurements (mm)	*Quantitative multi-state character*

Table 2.5 continued

	Character	Character states	Character classes
74	ratio of inner involucral bract length to width	average	*Quantitative multi-state character*
75	inner involucral bract apex spreading	0 = not, 1 = slightly, 2 = moderate, 3 = strongly	*Ordered qualitative multi-state character*
76	density of trichomes on abaxial surface of inner involucral bracts	0 = absent, 1 = sparse, 2 = moderate, 3 = dense	*Ordered qualitative multi-state character*
77	number of inner involucral bracts	average of 10	*Quantitative multi-state character*
78	total number of involucral bracts	average of 10	*Quantitative multi-state character*
79	number of receptacle scales per capitulum	average of 10	*Quantitative multi-state character*
80	receptacle scale length	average of 10 measurements (mm)	*Quantitative multi-state character*
81	receptacle scale width	average of 10 measurements (mm)	*Quantitative multi-state character*
82	ratio of receptacle scale length width	average	*Quantitative multi-state character*
83	total number of white tipped appendages	average of 10	*Quantitative multi-state character*
84	pappus length	average of 10 measurements (mm)	*Quantitative multi-state character*
85	pappus tip thickening	1 = slightly, 2 = moderate, 3 = very	*Ordered qualitative multi-state character*
86	achene length	average of 10 measurements (mm)	*Quantitative multi-state character*
87	achene width	average of 10 measurements (mm)	*Quantitative multi-state character*
88	ratio of achene length to achene width	average	*Quantitative multi-state character*
89	density of twin hairs on achene	0 = absent; 0.5 = occasional (1 of 3 achenes with hair); 1 = sparse; 2 = moderate; 3 = dense; 4 = very dense	*Ordered qualitative multi-state character*
90	density of glandular hairs on achene	0 = absent; 0.5 = occasional (1 of 3 achenes with glandular hair); 1 = sparse; 2 = moderate; 3 = dense; 4 = very dense	*Ordered qualitative multi-state character*
MC1	pappus width at tip	average of 30 measurements (µm)	*Quantitative multi-state character*
MC2	number of apical pappus cells	average of 30	*Quantitative multi-state character*
MC3	breadth of apical pappus cells	average of 30 measurements (µm)	*Quantitative multi-state character*
MC4	pappus width at widest part	average of 30 measurements (µm)	*Quantitative multi-state character*
MC5	pappus width at centre	average of 30 measurements (µm)	*Quantitative multi-state character*
MC6	length of barbellae	average of 30 measurements (µm)	*Quantitative multi-state character*
MC7	distance between barbellae and axis	average of 30 measurements (µm)	*Quantitative multi-state character*
MC8	= 89 re-assessed under compound microscope	0 = absent; 0.5 = occasional (1 of 3 achenes with hair); 1 = sparse; 2 = moderate; 3 = dense; 4 = very dense	*Ordered qualitative multi-state character*
MC9	= 90 re-assessed under compound microscope	0 = absent; 0.5 = occasional (1 of 3 achenes with glandular hair); 1 = sparse; 2 = moderate; 3 = dense; 4 = very dense	*Ordered qualitative multi-state character*
MC10	anther insertion point	average of 10 measurements (µm)	*Quantitative multi-state character*
MC11	= 61 re-assessed under compound microscope	average of 10 measurements (mm)	*Quantitative multi-state character*
MC12	ratio of corolla tube length to anther insertion point	average of 10 measurements (µm)	*Quantitative multi-state character*
MC13	anther connective base length	average of 10 measurements (µm)	*Quantitative multi-state character*
MC14	anther basal appendage length	average of 10 measurements (µm)	*Quantitative multi-state character*
MC15	ratio of anther connective base length to anther basal appendage length	average of 10 measurements (µm)	*Quantitative multi-state character*

2.3.2.1 Vegetative Morphology

Habit, growth form and general appearance

Ozothamnus in New Zealand is a much-branched shrub, lacking a distinct trunk. Five characters were chosen to describe differences in growth form and general appearance of these shrubs.

Three main growth forms (1) could be distinguished: 1) A generally "untidy" appearance with only a few (1-3) main branches. The new branchlets below the terminal inflorescence are often well developed while the flowers are still in bud. The shoots are long and slender, more or less fastigiate and sometimes descending or deflexed (Fig. 2.4); 2) a rounded "tidy" erect bush with several (>5) main branches. The new branchlets develop below the old terminal inflorescence after the flowers have fruited and died. The shoots are short and stout and never descending or deflexed (Fig. 2.5); 3) a more or less "tidy" bush with several (>5) main branches of which some, mostly the inner ones are upright and the outer ones are decumbent or prostrate. The new branchlets below the terminal inflorescence do not start to elongate before the flowers have fruited and died (Fig. 2.6). Two additional character states (character states 1.5 and 2.5) were necessary to accommodate intermediate growth forms.

Environmental factors exert a considerable influence on the growth form. This could be shown by cultivation under uniform conditions. The differences between field and cultivated material became quite obvious with plants being assessed with character state 3 in the field. The decumbent or prostrate growth form could be observed in extreme environments, such as unsheltered and unstable sand dunes ('Retorta' group) or above the tree line on mountain ridges on the west side of the Main Divide ('Vauvilliersii West Coast group'). In both habitats the plants must endure extreme wind conditions. Under cultivated conditions these plants lose much of the decumbent growth form and become increasingly upright (character state 2-2.5).

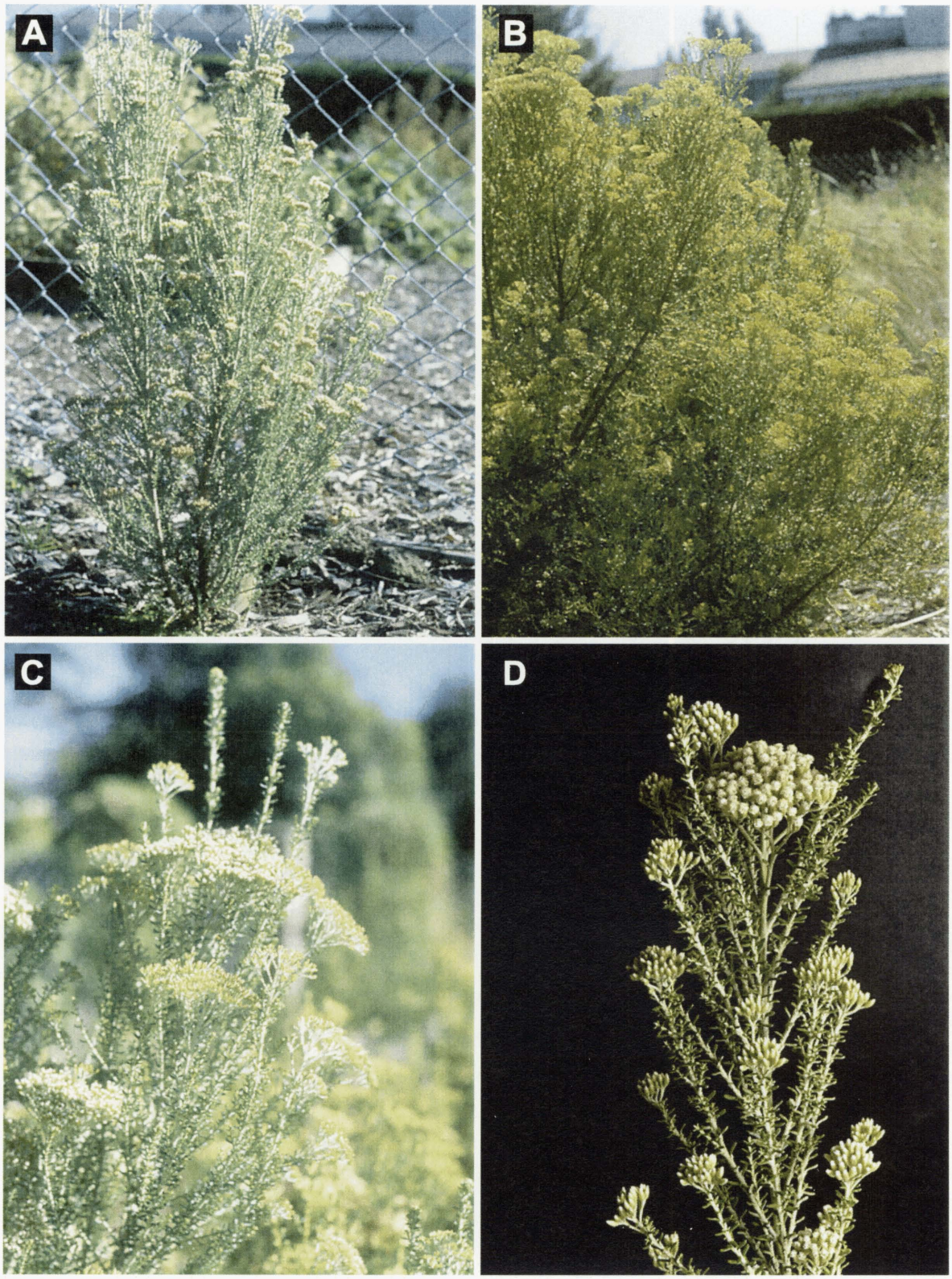


Fig. 2.4 Examples for character state 1 of "growth form" (character 1). (A) OTU 139A/fc, 'Leptophylla' group: upright, slender with long, slender, more or less fastigiate shoots; - (B) OTU 8G/fc, 'Fulvida Canterbury Coast' group: upright, slender with long shoots; - (C) OTU 58B/fc, 'Leptophylla' group: new shoots below the terminal inflorescence are well developed while the flowers are still in bud; - (D) OTU 70A/fc, 'Leptophylla' group: shoots below the terminal inflorescence well developed.
(D: photograph by M. Walters).

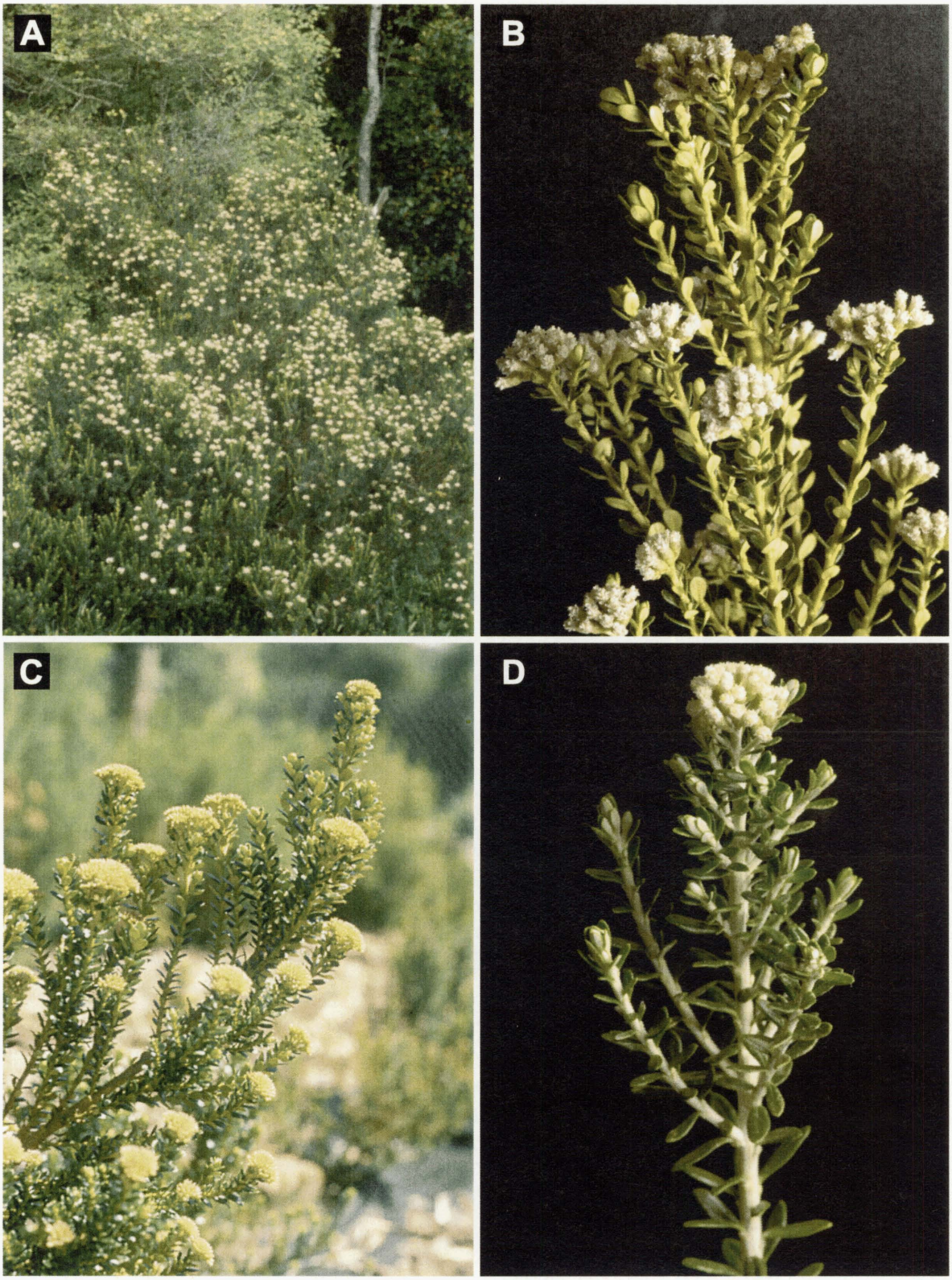


Fig. 2.5 Examples for character state 2 of "growth form" (character 1). (A) OTU 118A/ff, 'Vauvilliersii S-Otago/Southland/Fiordland' group: upright round, compact with stout shoots; - (B) OTU 17A/fc, 'Vauvilliersii S-Otago/Southland/Fiordland' group: shoots below the terminal inflorescence do not extend beyond the panicle; (C) OTU 16D/fc, 'Vauvilliersii S-Otago/Southland/Fiordland' group: shoots short and stout, not extending beyond the panicles; - (D) OTU 33B/fc, 'Vauvilliersii var. pallida/albida/canescens' group: shoots short and stout, not extending beyond the terminal inflorescence.
(B, D: photographs by M. Walters)

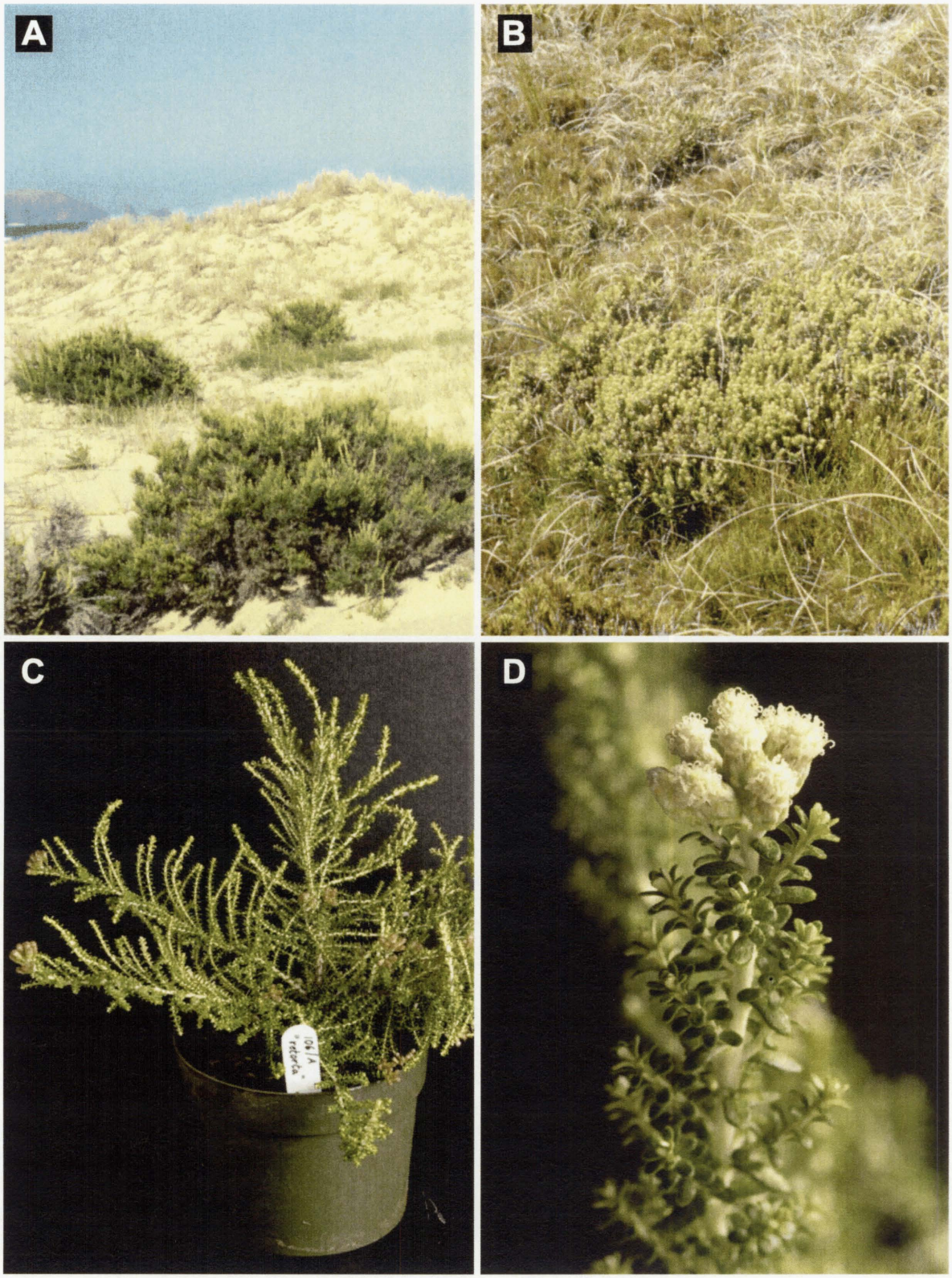


Fig. 2.6 Examples for character state 3 of "growth form" (character 1). (A) Population 105, 'Retorta' group, growing in sand dunes on Werahi Beach, Cape Reinga: plants with upright and prostrate shoots; - (B) OTU 9A/ff, 'Vauvilliersii West Coast' group, Garibaldi Ridge: plant with upright and prostrate short shoots; - (C) OTU 105A/fc, 'Retorta' group: inner shoots erect or upright, outer shoots decumbent or prostrate; - (D) OTU 105A/fc, 'Retorta' group: shoots not extending beyond the terminal inflorescence.
(B: photograph by A. Wilton; C, D: photographs by M. Walters)

Plant height (2) was measured from mature specimens (plants which have already reached flowering age), and coded as follows: 1 = not more than 80 cm, 2 = between 80 and 150 cm, 3 = over 150 cm. Specimens representing the 'Leptophylla' and 'Fulvida' groups reach the greatest heights. Most specimens of the 'Vauvilliersii' groups are between 80 and 150 cm tall, but some representatives of these groups, mainly specimens from the 'Vauvilliersii S-Otago/Southland/Fiordland' group, can reach a height of up to 3 m. Representatives of the 'Amoena' and the 'Vauvilliersii West Coast' group and some specimens of the 'Vauvilliersii var. pallida/albida/canescens' group are less than 80 cm tall.

Contributing to the general appearance of the plants are the angle between branchlet and branch or other branchlet (3), the diameter of the new season's growth, green branchlets (4), and the branchlet spacing (10) (number of branchlets per 5 cm of the new season growth). The average branchlet angle relative to the axis varies between 20.71° and 64.80° among specimens examined. The standard deviation within a single specimen ranges between ± 3.43 and ± 19.14, with an average standard deviation of ± 8.53 and a standard error of 0.23. Based on 10 measurements per specimen, this character is relatively variable within a single specimen. However, this character is of considerable value, as the variability is maintained in cultivation and it contributes towards the overall appearance of the plants and therefore each population as a whole. The branchlet angle is usually described in Floras as erect or spreading.

As compared with the branchlet angle, the branchlet diameter has a relatively small standard deviation (average stdev ± 0.26) within a single specimen, but considerable variation was noted between cultivated and field material. The branchlet diameter increases in cultivation under good light, water and nutrition conditions. A maximum of 3.06 mm and a minimum of 0.78 mm were measured for this character, which is described in Floras as stout or slender.

The density of the branchlets or the branchlet spacing (10) appears to be only slightly affected by the environmental conditions. Healthy growing cultivated specimens have longer shoots and the branchlets are less densely arranged. The number of branchlets per 5 cm of the branch ranges from 3.38 to 21.20. The average standard deviation within a single specimen is ± 2.30. Authors of Floras used the descriptive terms "densely branched", "much-branched" or "sparingly branched" to distinguish between groups of *Ozothamnus* (then species of *Cassinia*).

Indumentum of leaves and branchlets

An indumentum is always present on branchlets and leaves. Two main trichome types are present on the vegetative parts of all specimens examined. The first and dominant type is clothing trichomes, which are tricellular and consist of a bicellular, uniseriate pedestal (foot) and an unbranched, whip-like, more or less curly terminal cell. Each of the two foot cells is 17-20 μm long and 7-9 μm wide. The length of the terminal cell is difficult to determine because it tends to break easily due to its curliness and delicate structure. It is at least 2-3 mm on the abaxial surface of the leaves. The same type of clothing trichome occurs on the young branchlets and sometimes also on the adaxial surface of the leaves where it has a shorter less curly terminal cell.

No structural differences could be determined among the clothing trichomes in the *Ozothamnus leptophyllus* complex but the density and/or thickness of the tomentum may be an important taxonomic character. All of the specimens examined are tomentose on the abaxial surface of the leaves and on the young green branchlets, with a closely appressed, matted covering of trichomes. The indumentum thickness of the branchlet (5) and the indumentum thickness of the abaxial surface of young (30) and old (32) leaves varies between specimens and was assessed as sparse (character state 1) to extremely thick (character state 5) (Fig. 2.8).

The adaxial surface of the leaves is thinly tomentose (29, 31), but the trichomes are frequently shed from this surface. This varies not only between specimens but also between young (29) and old (31) leaves. Five character states were used to describe the trichome density of the adaxial leaf surface. This character includes the states 0 = trichomes are absent, 1 = trichomes sparse, almost no trichomes present, 2 = trichomes are thinly scattered, 3 = leaves more or less hairy, and 4 = tomentum very dense.

Differences in the assessment of the leaf indumentum of fresh and dry material are due to the fact that the trichomes are more obvious in fresh plants. This applies mainly to the adaxial surface of the leaves where the trichomes are difficult to observe even after dissecting. The leaf indumentum varies little between field and cultivated material.

The second type of trichome found on *Ozothamnus leptophyllus* leaves and branchlets is glandular. Glandular trichomes (Fig. 2.7: A-D) are present on the ad- and abaxial side of all *Ozothamnus* leaves examined. They were observed in very young leaves

where they occur very frequently among the developing non-glandular clothing trichomes. These glandular trichomes dry out in the mature leaves of nearly all specimens leaving the exudates from the glands between the clothing hairs. Just one group, the 'Vauvilliersii var. pallida/albida/canescens' group, has representatives with active glands on mature leaves. Fragments of the glandular trichomes are difficult to see beneath the fully developed clothing hairs and without care might be easily overlooked. The glandular hairs are composed of a multicellular biseriate stalk, terminated by a bicellular head (Fig. 2.7), with a total length of 0.06 - 0.1 mm. Little variation in the density, size and structure of the glandular trichomes was observed.

The degree of development of the indumentum did not appear to be correlated with altitude or climate, though further work may show it to be related to other ecological factors.

Leaf colour; exudate density and colour

The glandular trichomes of *Ozothamnus* exude several compounds (Webb 1988, Wood et. al 1999). As little variation in the density of glandular trichomes was observed, the variation in colour and stickiness of the leaves and young branchlets between specimens may be better understood through examination of their flavonoid profiles and the relative amounts of the compounds present. Flavonoids, mainly chalcones, dihydrochalcones and flavones (Wood 1992, Reid & Bohm 1994, Wood et al. 1999) have been isolated from the leaf exudate. Some of these possessed antiviral as well as antifungal activity (Wood 1992, Wood et. al 1999) (Section 1.4). Responsible for the more or less yellow or orange-yellow colour of leaves and branchlets are yellow quasi-crystalline deposits (Bohlmann et al. 1977, Haborne & Turner 1984) approximately 5 μm in length (Wood et al. 1999) that are formed by chalcones and 1'-(5'-acetyl-2'hydroxyphenyl)-3-methyl-2-buten-1-one.

In this study the exudates have been taken into consideration in so far as the colour of the adaxial (11, 12, 13, 14) and abaxial (15, 16, 17, 18) surface of the leaves, the exudate colour (7, 8, 33, 34, 36, 37, 39, 40, 42, 43) and density (6, 35, 38, 41, 44) and the stickiness of branchlets (9) and leaves (45, 46) were assessed and included in phenetic analyses as ordered multi-state characters.

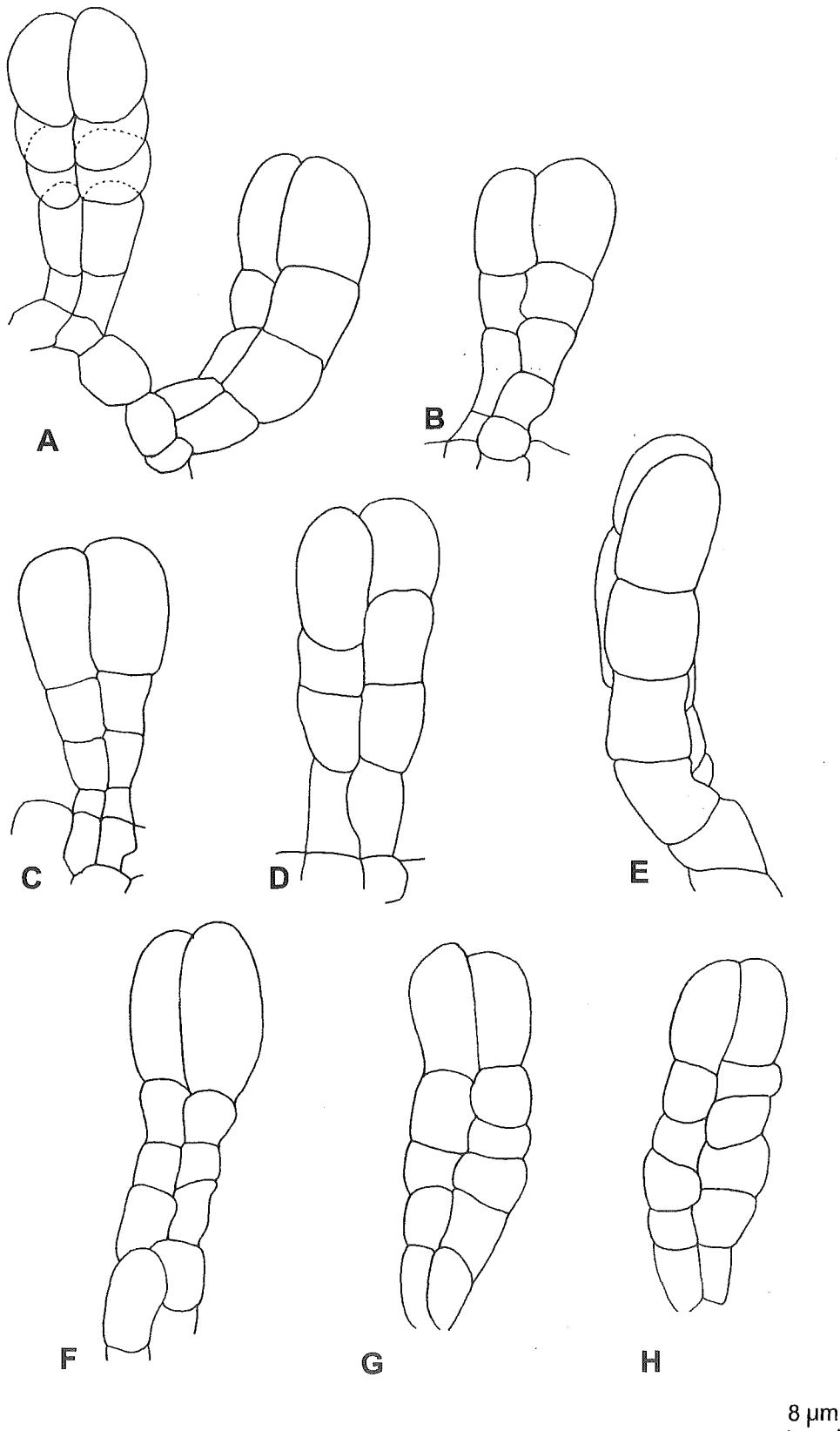


Fig. 2.7 Biseriate glandular hairs. (A) - (D): from very young leaves. (E), (F): from achenes. (G), (H): from corolla lobe. OTU 17A/fc, 'Vauvilliersii S-Otago/Southland/ Fiordland' group (A, E); OTU 7C/fc, 'Fulvida Canterbury Coast' group (B, G); OTU 106 A/fc, 'Retorta' group (C, F); OTU 33B/fc, 'Vauvilliersii var. pallida/albida/ canescens' group (D, H).

Leaf colour

The colour of the leaves was expressed by the colour gradient from "green to yellow" and the depth of the colour. Ten character states were distinguished (Appendix 5 Table 5) to describe the gradient, and seven character states were used to describe the depth of the colour.

The colour gradient of the adaxial surface of the leaves (11, 12) ranges from "dark yellowish green" to "greyish brown". The depth of the colour of the adaxial surface of the leaves (13, 14) ranges from "light yellow green" to "greyish brown" (Appendix 5 Table 6). Leaves become increasingly yellow and darker with age, so that the older leaves may have a different character state from the younger leaves.

The colour gradient of the abaxial surface of the leaves (15, 16) was assessed as "white/yellow green" (character state 1) to "strong orange yellow" (character state 7) (Appendix 5 Table 3). The colour depth of the abaxial surface of the leaves (17, 18) ranges from "white" (character state 1) to "deep yellow" (character state 5) (Appendix 5 Table 4). The colour of the abaxial surface of the leaves remains relatively constant between young and old leaves.

Exudate colour

Also assessed were exudate colours or the colour of the quasi-crystalline deposits on the young branchlets and both leaf surfaces of young (33, 34) and old (36, 37) leaves. The gradient from "green to yellow" (33, 36) ranged from "brilliant yellow green" (character state 1) to "strong orange yellow" (character state 7) (Appendix 5 Table 1). Three character states were distinguished for the depth of colour (34, 37). This character ranges also from "brilliant yellow green" (character state 1) to "strong orange yellow" (character state 3) (Appendix 5 Table 2).

The exudate colour depth (7) and gradient (8), of the branchlets are almost always the same as those of the abaxial surface of the young leaves (36, 37) and were excluded from further phenetic studies. There is little observable difference in the exudate colour between young (33, 34, 36, 37) and old leaves (39, 40, 42, 43) so the exudate colour characters for old leaves could also be eliminated from the data sets for phenetic analyses. The colour of the exudates changes little from fresh to dry material. Therefore exudate colours were included in addition to the leaf colours, which varies in 1-2 character states

between fresh and dry material. No variation for exudate colour was observed between field and cultivated material.

Differences in exudate density among the different groups are obvious. The exudate density on branchlets (6), ad- and abaxial surface of young (35, 38) and old (41, 44) leaves was assessed. It ranges from character state 0 = absent to 5 = extremely dense. Exudates on the adaxial surface of the leaves are less obvious. They are sparse or absent and more difficult to observe on the epidermis underneath the cuticle compared to the exudates in between the white clothing trichomes on the abaxial surface of the leaves.

Stickiness

Exudates are responsible for not only colour but also stickiness of branchlets (9) and leaves (45). These two characters were assessed with the states 0 = not to 3 = very strongly. The stickiness of the plants varies seasonally but some specimens remain glutinous throughout the year and stickiness can even be detected in the dry state. This phenomenon is described by character (46), a two-state (binary) character.

Other leaf characters

Generally description of the leaves:

The leaves of all *Ozothamnus* specimens studied are alternate, small, persistent, simple, entire and coriaceous. All leaves are narrowed to a very short erect petiole that is closely appressed to the branch. The lamina base is decurrent, the margins extending downwards along the petiole at a gradually decreasing angle to it.

Lamina orientation

The lamina orientation (19) contributes considerably to the general appearance of the plant and can be erect, suberect, or spreading. Old leaves on lignified stems often tend to spread to 90° or more in many populations. However, mature leaves on young branchlets often have a characteristic angle between the lamina and the branchlet. This was observed in cultivated and field material but it is difficult to assess this character using dry herbarium material. The angles between lamina and branchlets were recorded as absolute values and ranged from 36° to 89° with an average standard deviation of ± 19.37. The standard deviation within a single specimen ranges from ± 10.19 to ± 26.52.

Leaf shape and size (Fig. 2.8)

Three main leaf shapes were observed: 1 = oblong to narrow-oblong, 2 = elliptic to narrow-elliptic, 3 = obovate to narrow-obovate. It was common to observe two different leaf forms on a single specimen. Therefore two characters were required to describe the leaf form (20, 21). One leaf form is always dominant (main leaf form (20)), comprising approximately 80% of the leaves on a plant. Different representatives of a population sometimes vary in the distribution of leaf forms. The dominant leaf form in one specimen, may be the less common (or secondary) form (21) in another specimen of the same otherwise homogeneous population.

The leaf apex (22, 23) can be rounded = 0, obtuse = 1, acute = 2 or retuse = 3 (Fig. 2.8). To describe the leaf apex of a plant, two multi-state unordered characters were required. For a wide range of plants a main apex type, comprising approximately 80% of the leaves (22) and a less common apex type (23) can be distinguished.

The combination of rounded leaves and obtuse leaves occurs quite frequently within the 'Amoena' group (Fig. 2.8: A) and the 'Vauvilliersii S-Otago/Southland/ Fiordland' group. Representatives of these groups tend to have obovate leaves (Fig. 2.8: A, C). In the 'Vauvilliersii S-Otago/ Southland/Fiordland' group, rounded leaves are sometimes combined with retuse leaves (Fig. 2.8: C). A retuse apex was observed only in this group. Plants of the 'Vauvilliersii var. pallida/albida/canescens' group have obovate or elliptic leaves or a combination of both with mostly an acute apex (Fig. 2.8: E). Rounded and obtuse apices were also observed in this group. Representatives of the 'Leptophylla' group have oblong leaves sometimes combined with elliptic leaves as secondary leaf form (Fig. 2.8: D). Rounded leaf apices are the most common in this group but obtuse and acute leaves can also be found. Plants from the 'Fulvida Central Otago/Inland Canterbury' group have oblong to elliptic leaves with acute apices, while elliptic to oblong leaves with acute to obtuse apices are dominant within the 'Fulvida Canterbury Coast' group (Fig. 2.8: F). All specimens examined from the 'Retorta' group have obovate leaves with obtuse or rounded apices. The leaves of plants collected from higher altitudes ('Vauvilliersii West Coast' group, 'Vauvilliersii Cant./Otago/N-Southland' group, and 'Vauvilliersii N-Island' group) are either elliptic or obovate. The apices are usually obtuse or acute, but occasionally even rounded leaves could also be found in these groups.

Leaf size (lamina length (24), lamina width (25)), and the ratio length/width (26)

provide useful characters. The ratio length/width (26) allows distinction between oblong and narrow-oblong, elliptic and narrow-elliptic, obovate and narrow-obovate. Among specimens examined the average of the lamina length ranges from 2.30 mm to 13.18 mm, the lamina width from 0.97 mm to 4.78 mm. The average standard error of both, length and width is 0.15, indicating that these characters are quite consistent within one specimen. The leaf size varies slightly between field and cultivated material. Plants growing in cultivation tend to have slightly larger leaves than the same plants collected in the field.

Leaf margin (27) (Fig. 2.9)

While this character is found to vary between plants, it is consistent between field and cultivated material of the same plant. The level to which the margin is recurved is expressed as a single ordered multi-state character. The character states range from 1 = slightly to 4 = very strongly. Plants from coastal habitats ('Amoena' group (Fig. 2.9: B), 'Leptophylla' group (Fig. 2.9: G), 'Retorta' group (Fig. 2.9: F), 'Fulvida Canterbury Coast' (Fig. 2.9: H)) and from extremely dry habitats ('Fulvida Central Otago/Inland Canterbury' group (Fig. 2.9: E)) have strongly to very strongly recurved leaf margins, while those from populations at higher altitudes are often only slightly to moderate recurved ('Vauvilliersii' groups (Fig. 2.9: C, D)).

Midrib (28) (Fig. 2.9)

The leaf venation of *Ozothamnus* was not examined in detail. The midrib of the leaves of the specimens examined always protrudes abaxially. The amount of protrusion varies between populations. Leaf sections were made if the amount of protrusion was difficult to see under dense clothing trichomes (Fig. 2.9).

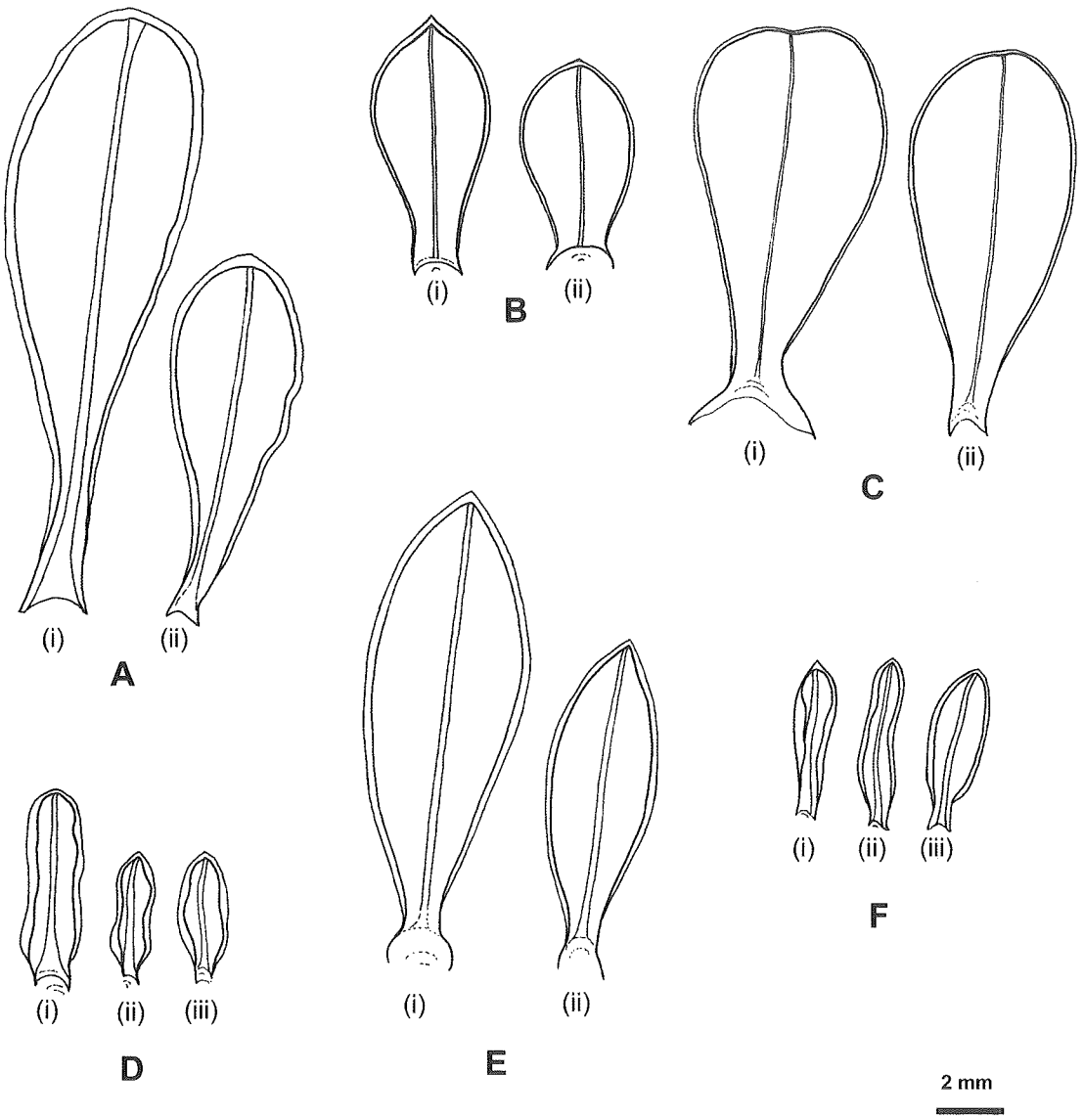


Fig. 2.8 Examples illustrating variation in leaf size, shape, and apex within the *O. leptophyllus* complex. Abaxial surface of leaves of (A) OTU 158/fc, 'Amoena' group, (i) obovate, rounded; (ii) obovate, obtuse; - (B) OTU 112A/fc, 'Vauvilliersii N-Island' group, (i) obovate, acute; (ii) obovate, acute; - (C) OTU 175A/ff, 'Vauvilliersii S-Otago/ Southland/Fiordland' group; (i) obovate, retuse; (ii) obovate, rounded; - (D) OTU 58A/fc, 'Leptophylla' group, (i) oblong, rounded; (ii) oblong, acute; (iii) elliptic, obtuse; - (E) OTU 33B/fc, 'Vauvilliersii var. pallida/albida/canescens' group, (i) obovate, acute; (ii) elliptic, acute; - (F) OTU 43A/fc, 'Fulvida Central Otago/Inland Canterbury' group, (i) elliptic, acute; (ii) oblong, acute; (iii) elliptic, obtuse.

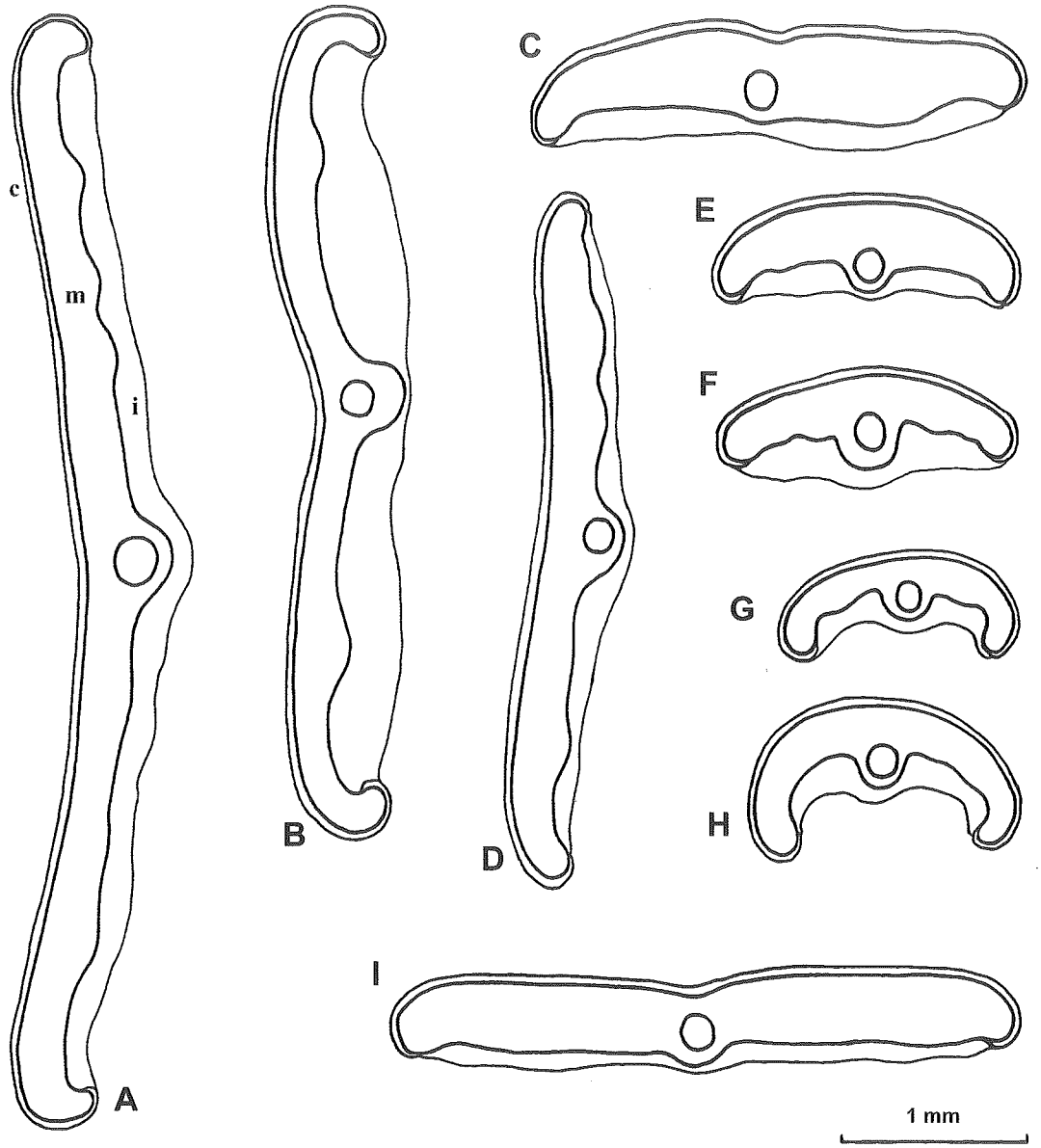


Fig. 2.9 Leaf cross sections. c = cuticle, m = mesophyll, i = indumentum. Illustration of differences in the form of leaf margins (Character 27), protrusion of midrib (Character 28), and thickness of the indumentum (Character 30).
(A) OTU 33B/fc, 'Vauvilliersii var. pallida/albida/canescens' group; leaf margin "strongly recurved", midrib "strongly evident", indumentum "very thick". - (B) OTU 158/fc, 'Amoena' group; leaf margin "very strongly recurved", midrib "very strongly evident" indumentum "extremely thick". - (C) OTU 13A/fc, 'Vauvilliersii West Coast' group; leaf margin "slightly recurved", midrib "slightly evident", indumentum "moderate". - (D) OTU 112A/fc, 'Vauvilliersii N-Island' group; leaf margin "moderate recurved", midrib "strongly evident", indumentum "moderate". - (E) OTU 34A/fc, 'Fulvida Central Otago/inland Canterbury' group; leaf margin "strongly recurved", midrib "strongly evident", indumentum "moderate". - (F) OTU 105B/fc, 'Retorta' group; leaf margin "strongly recurved", midrib "very strongly evident", indumentum "very thick". - (G) OTU 58B/fc, 'Leptophylla' group; leaf margin "very strongly recurved", midrib "very strongly evident", indumentum "very thick". - (H) OTU 7C/fc, 'Fulvida Canterbury Coast' group; leaf margin "very strongly recurved", midrib "very strongly evident", indumentum "very thick". - (I) OTU 17A/fc, 'Vauvilliersii S-Otago/Southland/Fiordland' group; leaf margin "slightly recurved", midrib "slightly evident", indumentum "sparse". Character states are enclosed in double quote marks.

Structure (52) (53), compactness (48), (51) and size (49), (50) of panicles

The overall appearance of the terminal flower clusters was expressed using six different characters. The structure of the panicles (52, 53) or the ramification pattern within the compound inflorescence varies between specimens. Plants with the capitula in a simple corymb ('Retorta' group) occur as well as plants with very complex panicles in which the 'primary' clusters are supported by ramifying peduncles of varying length. These peduncles are each subtended by a single scalelike 'bract' and show the same indumentum, exudate colour and density as the non-flowering branchlets of the plant. The number of ramification points within a panicle was used as a character to describe the complexity of the inflorescences. The majority of plants have 2 to 3 ramifications within a panicle. This number may rise up to 4 and 5 within the spreading panicles found within the 'Leptophylla' group. The ramification number is negatively correlated with the length of the first peduncle ('pedicel') (53). A similar general appearance to corymbs with a low ramification number and long pedicels is obtained by combining a high ramification number with short pedicels, as seen also in the 'Leptophylla' group. Both of these characters can vary within one population and even within one plant (refer to the standard error of 0.46 for the length of the pedicels (Appendix 7)).

The compactness of the panicle (48) was not found to vary within a plant. Four character states were used to describe the compactness of the panicle which usually consists of several corymbs: 1 = very spreading (Fig. 2.4: C), 2 = spreading (Fig. 2.4: D), 3 = dense (Fig. 2.5: B), 4 = very dense (Fig. 2.5: D). Representatives of the 'Leptophylla' and 'Fulvida' groups have usually more or less spreading panicles. To estimate the density of the panicles the diameter of the panicle was divided by the product of the number of capitula and the involucre diameter of the capitulum (51). The compactness of the panicle (48) is easily assessed and describes the general appearance of the panicle, while not necessarily reflecting the density of the capitula arrangement, (as it does character 51). This character is influenced by the size of the capitula, the state of ramification within a panicle, and the length of the pedicels. Characters 48 and 51 were shown to be independent and therefore both were included in analyses.

The size of the panicles was expressed by measuring their diameter (50) and by the number of capitula per panicle (49). Among all specimens examined, the diameter of the terminal clusters ranges from 7.75 to 70.33 mm. This character was found to vary only

slightly (standard error: 0.17) within a single specimen. The variation was greater among specimens within and among populations. In contrast, the number of capitula per terminal panicle is more variable within a single specimen (standard error: 0.28) but was relatively consistent within a population. Especially high capitula numbers per panicle (up to 300) are found within the 'Leptophylla' and the 'Fulvida' groups, while the specimens examined from the 'Retorta' group have only a few capitula (5-15) per panicle.

Capitulum (54-59)

The size of a capitulum can be expressed by measurements of the diameter or top width (54) and the length (55). The length of a capitulum can be as little as 3.83 mm within the 'Fulvida' groups and as much as 8.83 mm within the 'Retorta' group. The diameter of the capitulum ranges from 1.16 mm to 4.79 mm within the specimens included in this study. Both length and diameter of the capitula are highly consistent within a single specimen but vary depending upon the development stage of the capitulum. The diameter can also vary according to relative atmospheric humidity. This can make it difficult to take measurements of dry or rehydrated material, which would explain differences between fresh and herbarium material of the same plant. This also applies to involucre length (56) and capitulum body width (= involucre width) (57).

All capitula are more or less campanulate. The outer involucral bracts form a turbinate involucre. The inner, more or less radiating involucral bracts tower over this structure and give the capitulum a campanulate appearance. The ratio of capitulum body width to capitulum length (58) and the ratio of capitulum top width (diameter) to body width (59) add to the description of the shape of the capitula and involucres. Very wide capitula and involucres (high ratios of capitulum body width to capitulum length) can be found in all 'Vauvilliersii' groups, while representatives of the 'Leptophylla' and 'Fulvida' groups always have narrow capitula and involucres.

Involucral bracts and receptacle scales (67-83) (Fig. 2.10)

The involucral bracts occur in a compressed spiral, free and partly overlapping.
The shape (67) ranges from narrow-ovate to wide-ovate or narrow-oblong to oblong respectively within the range of specimens. The outer involucral bracts are never flat; the

lower parts are distinctly concave. The lower middle part of the outer involucral bracts is usually greenish brown; the edges are tawny, scarious, membranous, and the margins entire or torn. The tips are tawny scarious, or tawny with a flat white or cream scarious, erose or torn apex (Fig. 2.10: D, a-c; E: a-c). Some outer bracts are reddish towards the tips. Seven character states were chosen to describe the colour of the outer involucral bract tips (68), which is always consistent within a specimen but can vary between specimens from an otherwise homogeneous population. The outer involucral bracts usually radiate at the tip (69) and have some indumentum on the abaxial surface. The indumentum is denser towards the tips. The trichome types found on the bracts are clothing and glandular trichomes of the same structure as the trichomes found on the leaves and branchlets (refer to Indumentum of leaves and branchlets in 2.3.2.1). The density of the clothing trichomes on the outer involucral bracts (70) is expressed by 3 character states: 1 = sparse (Fig. 2.10: C, a-e), 2 = moderate (Fig. 2.10: B, a-e), 3 = dense (Fig. 2.10: A, a-c). Exudates can also be found on the abaxial surface of the bracts. The outer involucral bracts increase in size gradually from outside to inside and grade more or less gradually into the inner involucral bracts. The mean number of the outer involucral bracts ranges from 5.9 to 20 but is hardly variable within one specimen.

The inner involucral bracts are narrow-oblong to linear-oblong (74), slightly widening towards the white tips, and slightly concave at the base (Fig. 2.10). The average length varies little within a specimen and ranges from 2.82 mm to 6.22 mm within the total range of specimens. The width varies slightly more, even within one capitulum. The inner involucral bracts decrease in width from outside to inside until the innermost bracts have almost the same width as the receptacle scales. The average width ranges from 0.40 mm to 1.61 mm. The inner involucral bracts are membranous with scarious, entire or torn-ciliate margins, and white enlarged radiating tips of slightly variable size and shape with sometimes erose margins. Some inner bracts are reddish or yellowish towards the white radiating tips. The degree of spreading of the tips depends not only on the stage of development of the capitulum and the atmospheric relative humidity but also varies between specimens and populations (75). The inner involucral bracts are glabrous or nearly so to pubescent-ciliate (76). The number of inner involucral bracts (77) is, like the number of outer involucral bracts, quite variable between specimens of different populations. It ranges from 4.60 to 15.40.

Outer and inner involucral bracts blend into each other (Fig. 2.10), which makes it sometimes difficult to distinguish between them and to get an exact number. Therefore the total number of involucral bracts per capitulum (78) was assessed and found to range from 12.10 to 30.40 (standard error 0.07).

Not only outer and inner involucral bracts blend into each other but also the innermost inner involucral bracts and the receptacle scales have very similar size and appearance. While the length of receptacle scales (80) and the length of inner involucral bracts (72) are almost identical (the receptacle scale length ranges from 2.81 mm to 6.88 mm), the receptacle scales (81) are narrower than the inner involucral bracts, ranging from 0.28 mm to 0.82 mm. Occasionally the receptacle scales are folded in half, pleated or even keeled (Fig. 2.10: D, g and h). This is particularly obvious in specimens with many scales. The receptacle scales also have white tips, of similar size and shape to those of the inner involucral bracts, but the white tips of the receptacle scales radiate less. The average number of receptacle scales per capitulum (79) ranges from 0.00 to 26.90. Slight variation in the number of receptacle scales could be found in different capitula of a single specimen as well as in capitula of specimens within an otherwise homogeneous population. The largest numbers of receptacle scales were found within the 'Retorta' group. Difficulties in distinguishing between inner involucral bracts and receptacle scales made it necessary to use the total number of white tipped bracts/scales (83) as an additional character. This number ranges from 6.50 to 34.20.

All of the above capitula characters do not vary outside the range of the standard deviation between field and cultivated material.

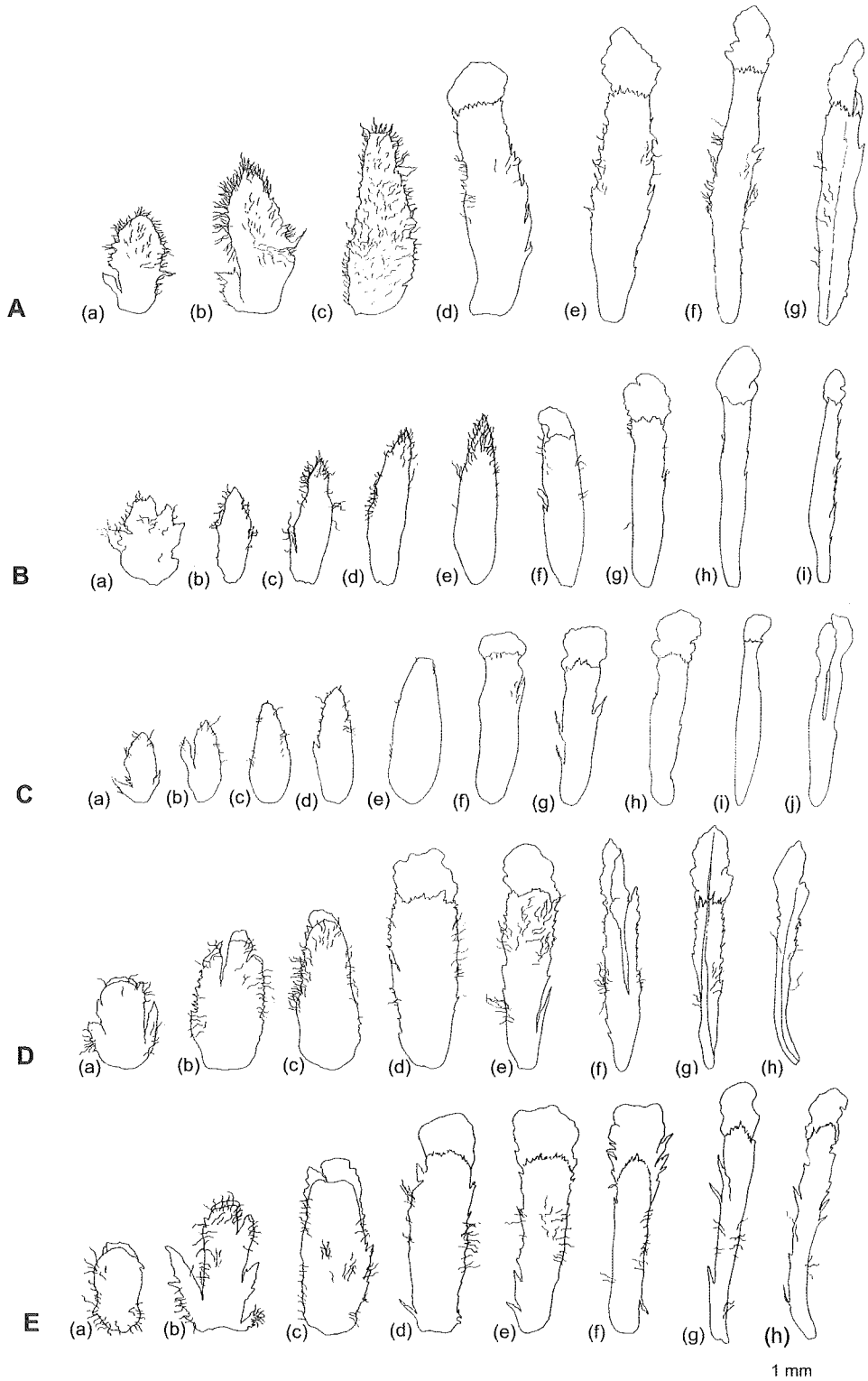


Fig. 2.10 Involucral bracts and receptacle scales (abaxial surface). (A) OTU 106A/fc, 'Retorta' group, (a)-(c) outer involucral bracts; (d), (e) inner involucral bracts; (f), (g) receptacle scales; - (B) OTU 70A/fc, 'Leptophylla' group, (a)-(e) outer involucral bracts; (f), (g) inner involucral bracts; (h), (i) receptacle scales; - (C) OTU 8G/fc, 'Fulvida Canterbury Coast' group, (a)-(e) outer involucral bracts; (f)-(h) inner involucral bracts; (i), (j) receptacle scales; - (D) OTU 65A/fc, 'Vauvilliersii var. pallida/albida/canescens' group, (a)-(c) outer involucral bracts; (d), (e) inner involucral bracts; (f)-(h) receptacle scales; - (E) OTU 17A/fc, 'Vauvilliersii S-Otago/Southland/Fiordland' group, (a)-(c) outer involucral bracts; (d)-(f) inner involucral bracts; (g), (h) receptacle scales.

Florets

All florets are perfect, gamopetalous and tubular except at the apex that is divided into five lobes. The corolla tube is cylindrical (continuing straight up with no dilation above the insertion of the filaments), but with usually a slightly expanded base. The triangular lobes are spreading to reflexed, a feature that varies both with stage of the floret development and among specimens. The corolla lobes are glandular. The glandular trichomes found on the corolla are identical to those of the leaves, involucral bracts and achenes (Fig. 2.7: G, H). The colour of the corolla is white to cream, or light brown in herbarium specimens.

The corolla varies in size among specimens. The corolla tube length was measured for each specimen (61), as well as the corolla lobe length (62) and width (63) and the maximum diameter of the corolla (64). The means of these corolla characters are relatively consistent within a single specimen. The corolla tube length ranges from 1.86 mm to 4.79 mm, the corolla lobe length from 0.39 mm to 0.96 mm, the lobe width from 0.21 mm to 0.50 mm and the maximum diameter of the corolla from 0.59 mm to 1.60 mm.

The floret number per capitulum (60) was found to be relatively consistent within a single specimen. Individuals of one population and even of one group have similar numbers of florets per capitulum. Many florets (up to 20) were found in capitula of representatives of 'Retorta' and of some 'Vauvilliersii' groups ('Vauvilliersii S-Otago/Southland/ Fiordland', 'Vauvilliersii var. pallida/albida/canescens'). Plants within the 'Fulvida' groups have only 4 to 7 florets per capitulum. Representatives of the 'Leptophylla' group have always slightly higher floret numbers per capitulum (from 9 to 15).

The style is always swollen or club-shaped at the base and forms a stylopodium. The style is bifurcate and divides towards the apex into two truncate, papillose and usually recurved arms (stigmatic lobes). The average length of the style (65) without the stigmatic lobes ranges from 1.64 mm to 4.67 mm. With a standard error of 0.07, this character is relatively consistent within a single specimen. The length of the style arms (66) varies slightly more within a single specimen (standard error 0.12), and ranges from 0.38 mm to 1.19 mm in total.

The anthers form a connate tube surrounding the style, and open longitudinally and introrsely. They are provided with apical and basal appendages. Each connective is projected into a sterile, flat and more or less triangular-acuminate appendage at the top.

These apical appendages are fairly uniform in size and shape among the specimens examined. The basal appendages, usually termed tails, are long, branch prolifically and extend beyond the connective base and filament interface. They vary in length (Microcharacter MC14) and range from 25.5 μm to 47.8 μm among the specimens examined. The length of the connective base (MC13) ranges from 19.0 μm to 30.40 μm and its length relative to that of the basal appendages (MC15) ranges from 0.61 μm to 0.86 μm among representatives of different groups (Appendix 7 Table 4).

The filaments are inserted in the corolla tube and attached to the base of the connective. The insertion point of the filament into the corolla varies. The distance between corolla base and insertion point (MC10) was measured and the ratio of corolla tube length and anther insertion point (MC12) calculated and found to be slightly variable within the range of specimens included in the microcharacter examination.

Pappus

The pappus hairs are in a single series and connate at the base. They are broad, flattened and expanded towards the tips. Each pappus hair is composed of 3-8 parallel rows of vertically elongated cells, which are coherent along some of their length. The upper part of the cells is free and projects at varying angles. The cells towards the apex of the pappus hair are clavate. The cells at the centre and at the base of the pappus hairs are apically produced into acute-acuminate lateral teeth. These teeth (barbellae) are approximately as long as the diameter of the pappus hair. The term barbellate is used to describe this type of pappus hair.

The average length of the pappus (84) was assessed and found to range from 2.01 mm to 5.01 mm. The extent to which the pappus hairs thicken towards the tips was assessed with an ordered multi-state character (85). The width of the pappus hairs at the tip (MC1), at the widest part of the hair (MC4) and at the centre (MC5) was found to vary slightly between representatives of different groups. The number of apical cells (MC2) is fairly consistent within and among specimens. The width of these cells (MC3) varies slightly between representatives of different groups. Differences in the length of the barbellae (MC6) and their angle (MC7) were also found between different specimens (Appendix 7 Table 4).

Achenes

The achenes are oblong, slightly compressed, and slightly narrowed to the base and vary in absolute size and presence of hairs. The length (86) ranges from 0.59 mm to 1.81 mm, the width (87) from 0.27 mm to 0.61 mm. Two different types of trichomes could be observed on the achenes: glandular trichomes and twin or duplex hairs. The glandular trichomes are identical in structure to those found on leaves, branchlets, involucral bracts and corolla lobes (Fig. 2.7: E, F). The twin hairs consist of four parallel cells, two basal and two elongated (Fig. 2.11). These hairs are antrorse and highly variable in length even on one achene. The densities of the achenial twin hairs (89) and glandular trichomes (90) were described with six character states each, ranging from trichomes absent to trichomes very dense.

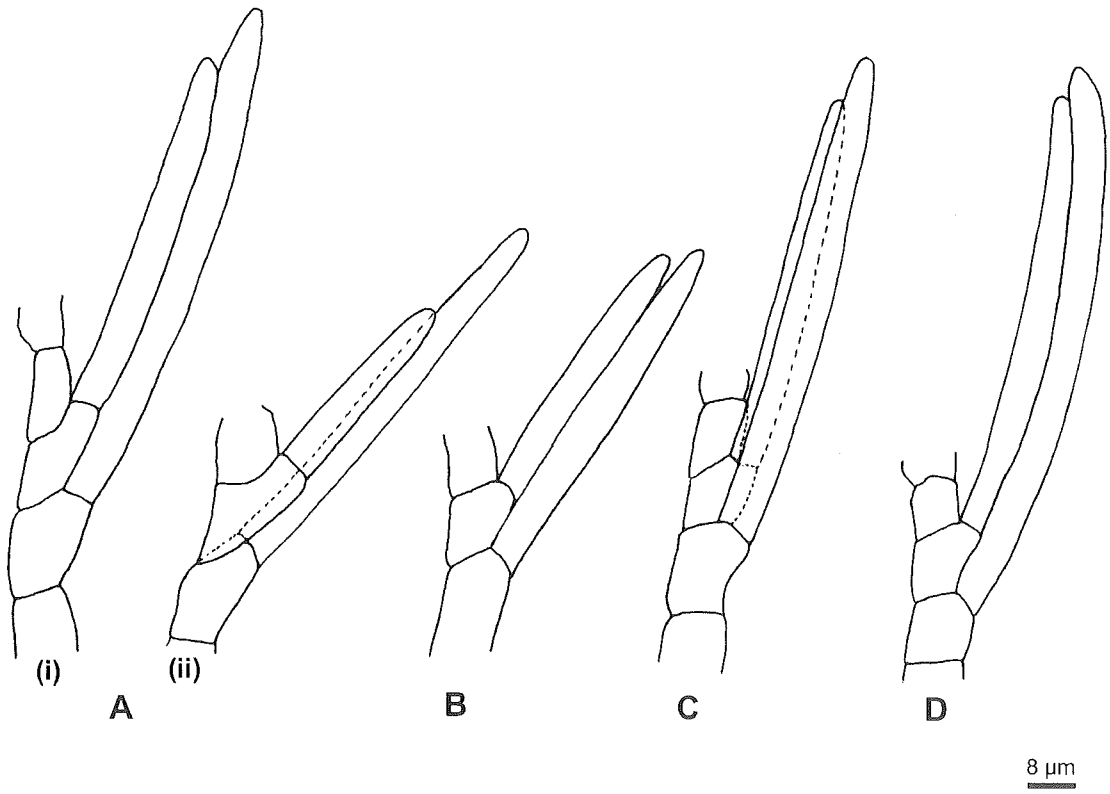


Fig. 2.11 Twin hairs from achenes. (A) OTU 65A/fc, 'Vauvilliersii var. pallida/albida/ canescens' group, (i) and (ii) from a single achene; - (B) OTU 17A/fc, Vauvilliersii S-Otago/ Southland/Fiordland' group; - (C) OTU 8 A/fc, 'Fulvida Canterbury Coast' group; - (D) OTU CHR355755 'Amoena' group.

External pollen morphology

Pollen grains are spheroidal and echinate with conspicuous spines. They are tricolporate, i.e., each pollen grain has three germinal apertures, and each surrounded by a groove or fold, termed a germinal furrow (colpus). The pores are circular to elliptical. The surface of the grain is covered by a continuous layer of exine (tectum). All specimens examined have tecta with many round perforations of similar size.

The most useful pollen characters identified in this study relate to the spines. Perforations are restricted to the lower half of each spine; the unperforated spine tip is smooth and all spines conical. However, spine characters (such as size, shape, gradient and density), are constant within, but variable among specimens. Most specimens have spine tips ranging between acute and subacute (e.g. OTU 139A/fc, Fig. 2.12: E; OTU 65A/fc, Fig. 2.12: A). Pollen with entirely subacute spines was also found (e.g. OTU 7C/fc, Fig. 2.12: D). Some specimens have pollen with subacute to obtuse spine tips (e.g. OTU CHR355755). Pollen with entirely obtuse spine tips was not present among the samples examined. The shortest spines were found in OTUs 65A/fc (Fig. 2.12: A), 64A/fc (Fig. 2.12: B), and 15A/fc, the longest in OTU 7C/fc (Fig. 2.12: D), OTU 8G/fc, OTU 106A/fc, OTU 105A/fc, and OTU 70A/fc. The spine width is correlated with the spine length and the spine density. Long spines are narrower and more densely arranged (e.g., OTU 7C/fc Fig. 2.12: D). The base of each spine is rounded in outline for all of specimens examined. The size of the interspinal area varies, i.e., it is small when 1) spines with narrow bases are densely arranged (e.g. OTU 7C/fc Fig. 2.12: D) or 2) if the pollen grain has spines with very wide bases (e.g. OTU 65A/fc Fig. 2.12: A). The outline of lateral spines varies from straight (e.g. OTU 65A/fc Fig. 2.12: A) to slightly concave (e.g. OTU 64A/fc Fig. 2.12: B, OTU 7C/fc Fig. 2.12: D) and the spine angle from steep (e.g. OTU 7C/fc Fig. 2.12: D) to shallow (e.g. OTU 64A/fc Fig. 2.12: B).

The pollen could be arranged in an almost continuous sequence of character variability. Distinct groups were determined within this sequence based on spine gradient, spine shape, spine density and shape of the spine tip. These groups are described as follows:

Group 1) The pollen has short, fairly densely arranged spines with a wide base. The spine tip is subacute to acute. The spine angle is shallow. The most distinguishing feature for this

group is the straight lateral outline of the spines (Fig. 2.12: A). These features apply to pollen grains of OTU 33B/fc and OTU 65A/fc.

Group 2) The spines are also relatively short with a wide base and subacute to acute tips, but with a larger interspinal area between them. The spine angle is like that in the pollen grains of Group 1, i.e., shallow but the lateral outline of the spines is slightly concave. Refer to Fig. 2.12: B. Two subgroups could be determined based on the spine tips, i.e., pollen of OTU 64A/fc, OTU 13A/fc, and OTU 112 A/fc with acute to subacute spine tips, and pollen of OTU 16A/fc, 17A/fc, 118A/fc, and CHR355755 with nearly obtuse spine tips.

Group 3) The pollen of the third group is distinguished from Group 2 by slightly longer and narrower spines. The density of the spines was higher and the spine angle steeper. Refer to Fig. 2.12: C. Group 3 contains pollen of OTU 34A/fc, OTU 43A/fc, OTU 15A/fc, and OTU 4X/fc, and can be seen as an intermediate group between group 2 and 4.

Group 4) This group contains pollen with long narrow spines, subacute tips, and concave lateral outlines. The spines are densely arranged with a steep angle. Refer to Fig. 2.12: D. The pollen grains of the following specimen fall into this group: OTU 7C/fc, OTU 8G/fc, OTU 58B/fc, OTU 70A/fc, OTU 139B/fc, OTU 105A/fc, and OTU 106A/fc.

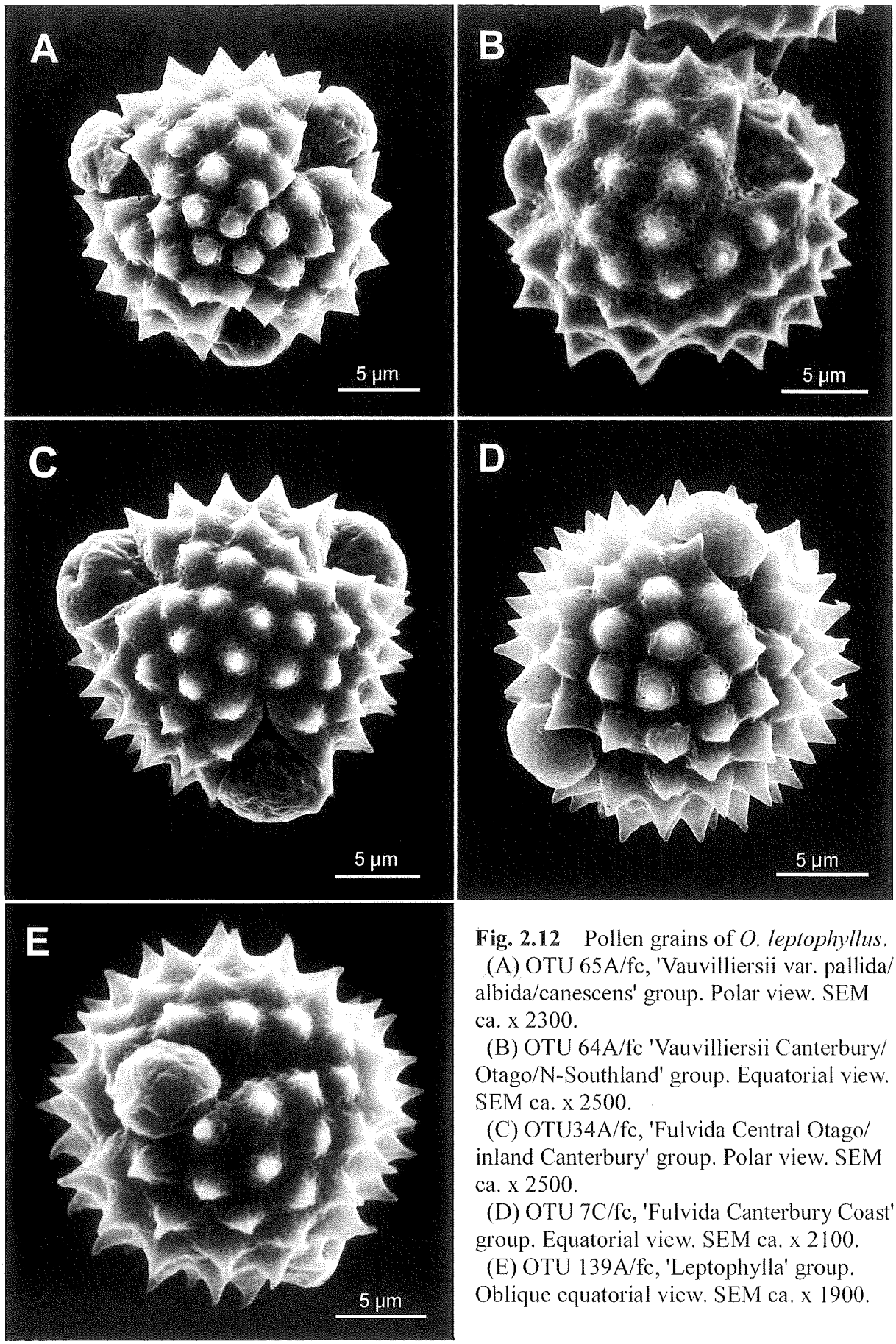


Fig. 2.12 Pollen grains of *O. leptophyllus*.
(A) OTU 65A/fc, 'Vauvilliersii var. pallida/albida/canescens' group. Polar view. SEM ca. x 2300.
(B) OTU 64A/fc 'Vauvilliersii Canterbury/Otago/N-Southland' group. Equatorial view. SEM ca. x 2500.
(C) OTU34A/fc, 'Fulvida Central Otago/inland Canterbury' group. Polar view. SEM ca. x 2500.
(D) OTU 7C/fc, 'Fulvida Canterbury Coast' group. Equatorial view. SEM ca. x 2100.
(E) OTU 139A/fc, 'Leptophylla' group. Oblique equatorial view. SEM ca. x 1900.

2.3.3 Numerical analyses

2.3.3.1 Numerical analyses of Data Sets 1-3

Numerical analyses of Data Set 1:

Data Set 1 contained all OTUs for which morphology had been examined, and 80 morphological characters (Table 2.5). The similarity matrix is shown in Appendix 8, the phenograms resulting from numerical analyses of Data Set 1 are shown in Fig. 2.13 and Appendix 9 Figs 1-3. In general, the different cluster analyses produced similar results. However, the hierarchical structure of the phenograms and the cophenetic correlation coefficients were used to select a phenogram to base the reduction of the number of OTUs on. The overall cophenetic correlation coefficients were as shown in Table 2.6.

Table 2.6 Cophenetic correlations of the phenograms based on Data Set 1 with the similarity matrix from which they are derived.

	Overall Cophenetic Correlation Coefficient	
	Pearson	Spearman Rank
Average Linkage (UPGMA) Phenogram	0.75081	0.74358
Single Linkage Phenogram	0.56371	0.5407
Complete Linkage Phenogram	0.64717	0.63131
Weighted (WPGMA) Phenogram	0.71656	0.69329

Clusters of highly similar OTUs were determined from the UPGMA phenogram, the phenogram with the highest overall cophenetic correlation coefficient. Twenty-one pairs of OTUs joined at a similarity level > 0.9. Fourteen clusters with three OTUs, one 4-OTU-cluster, one 5- two 6- and one 12-OTU-cluster occurred between 0.9 and 1.0. Each of these clusters, linking at a high level of similarity, contained both field and cultivated material of the same phenotype and/or different specimens from individual populations. These same clusters were present in the WPGMA phenogram. The single linkage method led to the formation of relatively larger clusters and produced a 'chained' topology. However, less clustering could be observed between a similarity of 0.9 and 1.0. By creating chains, two dissimilar sites were placed into the same cluster (linked by a chain of sites between them).

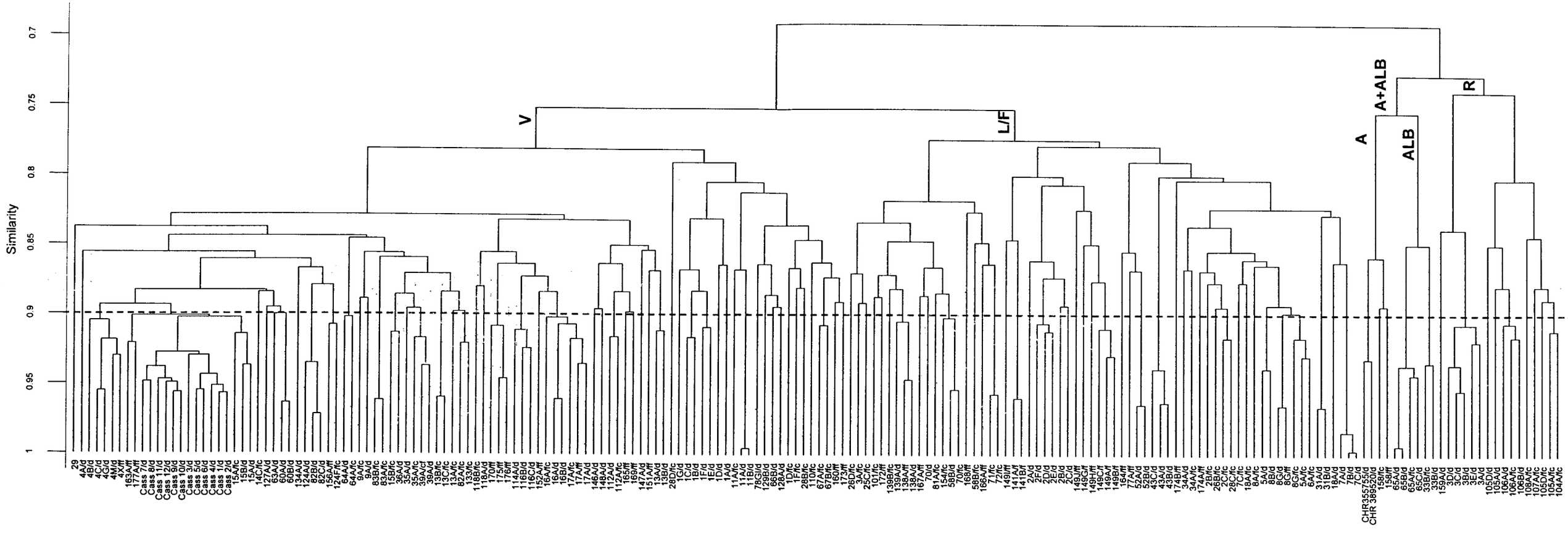


Fig. 2.13 UPGMA phenogram based on Data Set 1.

Some clusters observed in the UPGMA and WPGMA phenograms also occurred in the single linkage phenogram. The clusters produced by complete linkage clustering at a high similarity level were only slightly different to the ones created by the average linkage methods. Based on the UPGMA phenogram, a single OTU was chosen from each cluster which had formed at a similarity level > 0.9. These OTUs were then included in further analysis. This reduced the number of OTUs from 192 to 112.

Numerical analyses of Data Set 2:

Data Set 2 contained the reduced number of 112 OTUs (see above) and 80 morphological characters (Table 2.5). Numerical analysis performed on Data Set 2 (similarity matrix: Appendix 8) resulted again in four phenograms (Fig. 2.14, Appendix 10), that, due to the reduction of OTUs, were clearer and easier to interpret than those based on analyses of Data Set 1. The phenograms were compared based on the overall cophenetic correlation coefficient (Table 2.7) and the hierarchical structure. Based on this comparison, the UPGMA phenogram was chosen to define and describe clusters.

Table 2.7 Cophenetic correlations of the phenograms based on Data Set 2 with the similarity matrix from which they are derived.

	Overall Cophenetic Correlation Coefficient	
	Pearson	Spearman Rank
Average Linkage (UPGMA) Phenogram	0.71347	0.70139
Single Linkage Phenogram	0.60637	0.54210
Complete Linkage Phenogram	0.60564	0.59968
Weighted (WPGMA) Phenogram	0.60612	0.56863

The average linkage phenogram (Fig. 2.14) was split into four main clusters: **V** = '**V**auvilliersii', **L/F** = '**L**eptophylla/**F**ulvida', **A+ALB** = '**A**moena/**Alb**ida', **R** = '**R**etorta'.

Cluster V joined cluster L/F at 0.741, cluster A+ALB and cluster R linked at 0.711. Cluster pair V-L/F and cluster pair A+ALB-R joined at 0.687.

Cluster V was composed of two smaller subclusters (**Vau** = '**Vau**villiersii' and **VP/PP** = '**V**auvilliersii var. **p**allida' plus **p**olymorphic **p**opulations). The two subclusters were joined at a similarity level of 0.775. Each of the subclusters split further into three clusters (**VOSF**, **VCOS/VWC**, **VN** and **PP1**, **VP**, **PP2**). Cluster **VOSF** contained the specimens of the '**V**auvilliersii S-**O**tago/**S**outhland/**F**iordland' group and joined the cluster

pair **VCOS/VWC** and **VN** at 0.814. Cluster **VCOS/VWC** comprised specimens from the '**V**auvilliersii **C**ant./**O**tago/N-**S**outhland' and the '**V**auvilliersii **W**est **C**oast group'. Cluster **VCOS/VWC** and cluster **VN**, which contained mainly specimens of the '**V**auvilliersii **N**orth Island' group linked together at 0.82. The cluster VP/PP was composed of 3 subclusters, VP, PP1 and PP2. Cluster VP comprised specimens of the 'Vauvilliersii var. pallida/albida/canescens' group and linked together with cluster PP2 at 0.799. Cluster PP1 joined this cluster pair at 0.796. Cluster PP1 and PP2 contained exclusively big leaved specimens from polymorphic populations. Cluster PP1 was composed of specimens from only one population.

Cluster L/F was composed of three subclusters (**L, F,** and **PP3**). Cluster **L** contained the '**L**eptophylla' group and linked together with cluster **F**, the '**F**ulvida' cluster, at 0.775. The third subcluster (**PP3**) was formed by small leaved representatives of polymorphic populations and joined the linked subcluster L and F at 0.775. Subcluster F split at a similarity level of 0.784 into two clusters (**y** = **y**ellow, **o** = **o**range). The majority of the representatives of the 'Fulvida' fell into cluster y. Cluster o contained four OTUs with extremely dark yellow to orange exudates as a distinguishing feature.

Cluster R was formed by specimens from the 'Retorta' group. Besides specimens from the '**A**moena' group (Cluster **A**), cluster A+ALB contained representatives of the 'Vauvilliersii var. albida' group (cluster **ALB**).

The single linkage, complete linkage and WPGMA phenograms can be found in Appendix 10. The four main clusters (V, L/F, A and R) were present in all of the phenograms. Only minor differences were found in how these clusters linked together. In the WPGMA phenogram (Appendix 10 Fig. 3) the arrangement of the four main clusters was similar to that in the UPGMA phenogram. The only difference was that cluster V and cluster L/F joined at a slightly lower level of similarity (at 0.714). The single linkage phenogram (Appendix 10 Fig. 1) appeared to differ from the other phenograms mainly due to the large number of OTUs in isolated positions and the chaining effect. However, the same four main clusters were also present. In the complete linkage phenogram (Appendix 10 Fig. 2) clusters A and R joined at 0.625 and linked with cluster V at 0.5, before joining with cluster L/F at 0.458.

Fig. 2.14 UPGMA phenogram from Data Set 2.

The clusters of lower hierarchical order, observed in the UPGMA phenogram (Fig. 2.14) were also more or less obvious in nearly all of the phenograms. However their arrangements varied. Clusters including or composed of representatives of polymorphic populations (cluster PP1, cluster PP2, and cluster PP3) were often divided and rearranged by using different clustering methods.

In the WPGMA phenogram (Appendix 10 Fig. 3) cluster PP1 and cluster PP2 formed a subcluster together with a part of cluster PP3 within the L/F cluster. The remaining OTUs from cluster PP3 in the UPGMA phenogram were arranged in cluster F, the 'Fulvida' cluster. An affinity was indicated between representatives of the cluster VP ('Vauvilliersii var. pallida') and representatives of the cluster ALB by the linking these two clusters together within cluster A+ALB in the WPGMA phenogram.

In the complete linkage and the single linkage phenograms the representatives of polymorphic populations were widely separated into small clusters. In the complete linkage phenogram (Appendix 10 Fig. 2) small PP clusters could be found within subcluster VP ('Vauvilliersii var. pallida'), subcluster L ('Leptophylla') and subcluster F ('Fulvida'). In the single linkage phenogram (Appendix 10 Fig. 2) the representatives of the polymorphic populations were scattered mainly within the L/F ('Leptophylla/Fulvida') cluster, which was not split into a 'Leptophylla' and a 'Fulvida' cluster.

Display of intermediate groups and putative hybrids with principal coordinate analysis

The first three axes of the PCO explained nearly 32% of the variation in Data Set 2, with each axis explaining 14.4%, 11.1% and 6.5% respectively. Eleven clusters were depicted in the PCO plots (Fig. 2.15 - Fig. 2.17).

Principal coordinate 1 primarily distinguished three groups: 1) the 'Vauvilliersii' group, 2) the 'Fulvida' group together with the 'Vauvilliersii var. pallida' group, and 3) the 'Amoena', 'Albida', 'Leptophylla' and 'Retorta' groups. The second and third groups overlapped to some degree. Principal coordinate 2 separated the 'Retorta', 'Amoena' and 'Albida' groups from the 'Fulvida', 'Leptophylla' and 'Vauvilliersii' groups. The 'Vauvilliersii var. pallida' group occupied an intermediate position. Principal coordinate 3 separated the 'Retorta' group from other OTUs, and distinguished two other groups, one consisting of the 'Vauvilliersii var. pallida' and 'Albida' groups, and the other composed of the 'Leptophylla', 'Fulvida' and 'Vauvilliersii' groups.

OTUs 3B/d, 3A/fc, 26B/fc, 26D/fc, and 25C/fc originated from the same polymorphic population (Poly1) located at Ward beach. In the UPGMA cluster analysis these OTUs appeared in three separate clusters (PP3, F, and ALB). In the principal coordinate analysis three of these OTUs (3A/fc, 3B/d, and 26D/fc) clustered near the representatives of the 'Leptophylla' group; one OTU (26B/fc) was placed within the 'Fulvida' group and OTU 26D/fc occupied an intermediate position. Intermediate positions were also occupied by some representatives of the polymorphic population (Poly2), located in Marlborough on the Chalk Range (OTUs 1G/d, 1F/fc, 1E/d, 1D/fc, 1D/d, 1B/d, 1A/d) and below, from the Remuera Station from different altitudes (2A/d, 2B/fc, 2B/d, 2C/d, 2D/d, 28B/fc, 28C/fc, 28D/fc, 29). Most OTUs from the Remuera Station showed strong affinities to the 'Vauvilliersii var. pallida' group while others, especially OTUs from lower altitudes (OTUs 2A/d, 2B/fc, 2B/d, 2C/d) tended to be closer to the 'Fulvida' group or the 'Leptophylla' group. Only one representative of a polymorphic population from the Parara Wetland, Marlborough was included in this analysis (Poly3: OTU 141A/f). It occupied an intermediate position between the 'Leptophylla', 'Fulvida' and 'Vauvilliersii var. pallida' groups (Figs 2.15-2.17). Four of the five representatives of Poly4 (Isis Stream valley, Marlborough) showed strong affinities to one or sometimes two of the other groups. OTU 149I/ff was placed very close to the 'Vauvilliersii var. pallida' group (principal coordinate 1 and 2, Figs 2.15-2.17). Principal coordinate 3 (Figs 2.16, 2.17) placed this OTU between the 'Vauvilliersii var. pallida' and 'Vauvilliersii' groups. OTU 149H/ff showed a strong affinity to the 'Fulvida' group, while OTU 149G/ff was placed close to the 'Leptophylla' group (supported by all the principal coordinates, Figs 2.15-2.17). OTU 149J/ff also showed affinities to the 'Leptophylla' group (principal coordinate 1 and 2, Figs 2.15-2.17), but was placed close to representatives of Poly2 based on principal coordinate 3 (Figs 2.16, 2.17). OTU 149A/ff occupied an intermediate position between the 'Leptophylla', 'Fulvida', 'Vauvilliersii var. pallida' and the 'Vauvilliersii' groups. From the polymorphic population (Poly5), located at the Hodder River, Inland Kaikoura, Marlborough, one of the two representatives (OTU 174B/ff) was placed in the 'Fulvida' group. OTU 174A/ff, while also close to the 'Fulvida' group, tended to show some association with the 'Vauvilliersii var. pallida' group (principal coordinate 3 in Figs 2.16 and 2.17).

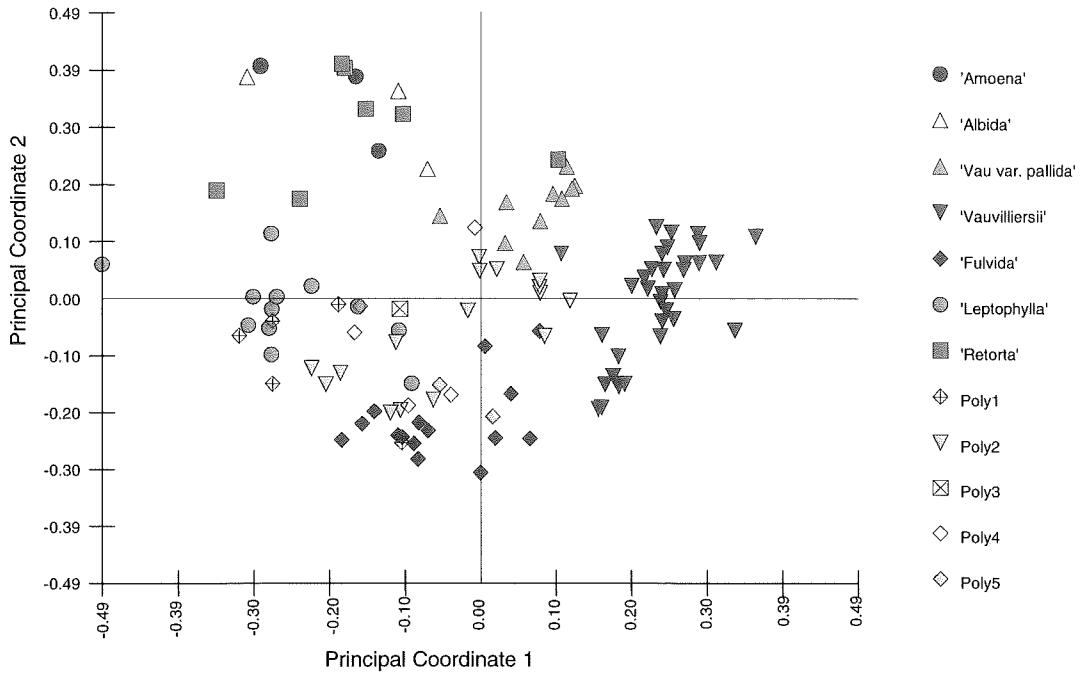


Fig. 2.15 Plot of principal coordinate analysis 1 vs. 2 generated from Gower's General Coefficient of Similarity based on Data Set 2.

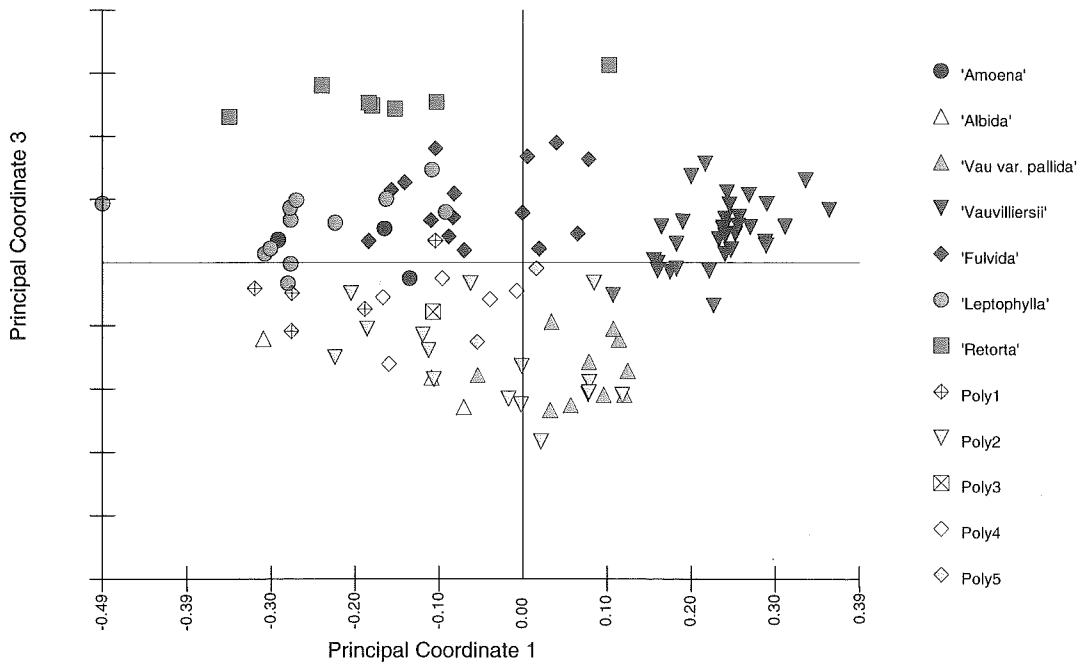


Fig. 2.16 Plot of principal coordinate analysis 1 vs. 3 generated from Gower's General Coefficient of Similarity based on Data Set 2.

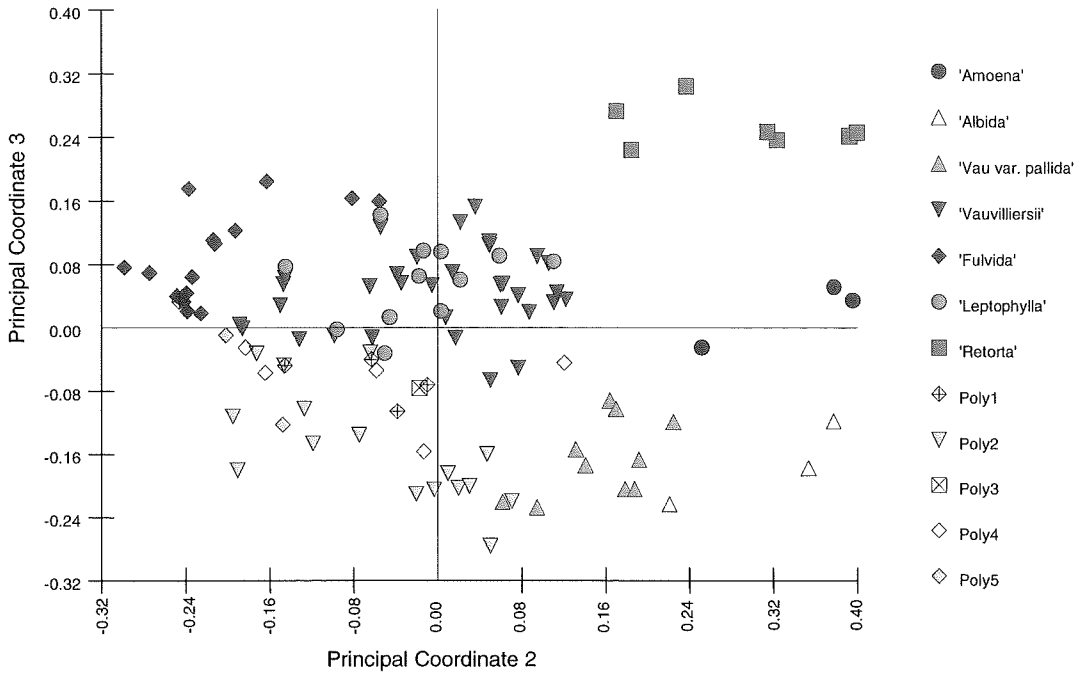


Fig. 2.17 Plot of principal coordinate analysis 2 vs. 3 generated from Gower's General Coefficient of Similarity based on Data Set 2.

Numerical analyses of Data Set 3:

Data Set 3 without the above OTUs contained 83 OTUs and 80 morphological characters (Table 2.5). The phenograms (Figs. 2.18 - 2.21) derived from the similarity matrix (Appendix 8) of Data Set 3 showed a similar structure to the ones created with Data Set 1 and 2. The overall cophenetic correlation coefficients were as shown in Table 2.8.

Table 2.1 Cophenetic correlations of the phenograms based on Data Set 3 with the similarity matrix from which they are derived.

	Overall Cophenetic Correlation Coefficient	
	Pearson	Spearman Rank
Average Linkage (UPGMA) Phenogram	0.75003	0.74186
Single Linkage Phenogram	0.66466	0.62906
Complete Linkage Phenogram	0.63592	0.52017
Weighted (WPGMA) Phenogram	0.67839	0.62250

The UPGMA phenogram (Fig. 2.18) was the phenogram with the highest overall cophenetic correlation coefficient. It was composed of the same four main clusters already

seen in phenograms derived from Data Sets 1 and 2 joined here at 0.668. Cluster V and cluster L/F joined at 0.718, and cluster A and cluster R joined at an only slightly lower level of similarity (0.714).

Cluster L/F was composed of the 'Leptophylla' cluster (L) (containing exclusively representatives of the 'Leptophylla' group) and the 'Fulvida' cluster (F) (formed by representatives of the two 'Fulvida' groups) (Section 2.3.1). Cluster F was split at 0.78 into two clusters y and o. The smaller cluster o contained four specimens, with a distinct appearance due to their orange or dark yellow colour of leaves and branchlets, a very strongly recurved leaf margin, small panicles, and slightly larger leaves. Three specimens in this cluster were from coastal habitats (164/ff, 52B/d, and 77A/ff) and joined together at a high level of similarity (0.863 and 0.852). OTU 43A/d from Central Otago joined this cluster at 0.79. Cluster y was formed by small leaved specimens from low altitudes close to the coast, which do not grow in coastal habitats (sand dunes or coastal rocks). Specimen 34A (dry field and fresh cultivated material linked at 0.855), the only inland specimen in this cluster, was the last to join at 0.814.

The second main cluster V comprised all the big leaved specimens included in this study (except for the specimens from the 'Amoena' group). Cluster V was formed by cluster Vau and cluster VP/ALB linking together at 0.745. Cluster VP/ALB was clearly divided into 2 clusters. Both contained specimens from the 'Vauvilliersii var. pallida/albida/canescens' group. The specimens forming the smaller cluster ALB (65A/fc, 159A/d and 33B/fc) could be found in the phenograms derived from Data Set 1 and 2 within the A+ALB cluster. The remaining specimens from the 'Vauvilliersii var. pallida/albida/canescens' group formed cluster VP. Cluster VP and cluster ALB joined together at 0.776. Cluster Vau was formed by clusters VCOS/VWC and VN (linked together at 0.812) linking with cluster VOSF at 0.815. Cluster A and cluster R contained specimens from the 'Amoena' group and 'Retorta' group respectively. A and R joined at 0.714.

The most remarkable difference between the UPGMA and the WPGMA phenogram (Fig. 2.21) was the positioning of cluster VP/ALB. In the WPGMA phenogram this cluster was linked together with the 'Amoena' cluster A at 0.728, while it represented a subcluster within the V cluster in the UPGMA phenogram. The Specimen 159A/d placed in the UPGMA phenogram within cluster ALB appeared in the WPGMA phenogram

within the 'Retorta' cluster R.

In the single linkage phenogram (Fig. 2.19) the OTUs 33B/fc and 65A/fc from the 'Vauvilliersii var. pallida/albida/canescens' group were isolated and were the last OTUs to be clustered at 0.8 and 0.806 respectively. The 'Amoena' cluster A also joined the phenogram at a low level of similarity (0.814), followed at 0.826 by another isolated OTU, 108A/fc from the 'Retorta' group. The remaining specimens from the 'Retorta' group formed cluster R and joined at 0.8269 a large cluster which could be seen as a combined cluster L/F and V. The largest cluster within the cluster L/F-V was cluster Vau which was linked together with the 'Fulvida' cluster F at 0.845. The pair formed by OTU 34A/d and 34A/fc was isolated and joined at 0.843 before the 'Leptophylla' cluster L joined at 0.841. Representatives of the 'Fulvida' cluster (o) in the UPGMA phenogram, these with conspicuous dark yellow/orange exudates were isolated from the rest of the 'Fulvida' cluster and joined at 0.84. Cluster Vau did not split into the clusters VOSF, VCOS/VWC and VN, which could be observed in the UPGMA and the WPGMA phenogram.

Most of the previously observed clusters could be found in the complete linkage phenogram (Fig. 2.20). As seen in the WPGMA and the single linkage phenogram was the cluster VP/ALB isolated from cluster Vau and linked together at 0.57 with cluster R and A. Cluster L/F was composed of cluster L and cluster F. The dark yellow/orange representatives of the 'Fulvida' cluster (o) were isolated from the rest of the 'Fulvida' cluster and grouped together within cluster Vau.

Based on a phenon line placed at the similarity level of 0.83 within the UPGMA phenogram, Data Set 3 was further reduced. Representatives of groups clustering together at or above this similarity level were chosen for further analysis as Data Set 4.

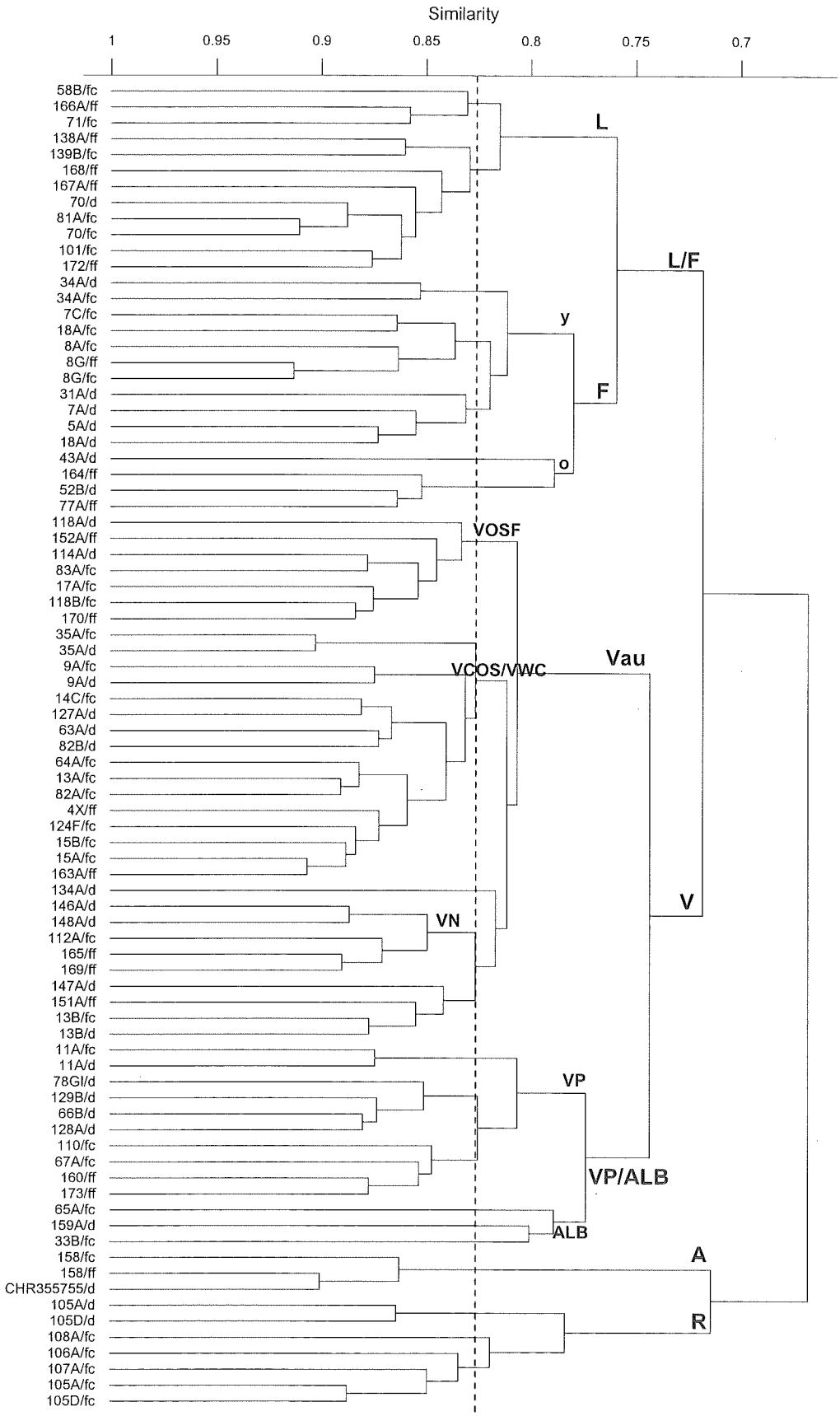


Fig. 2.18 UPGMA phenogram based on Data Set 3.

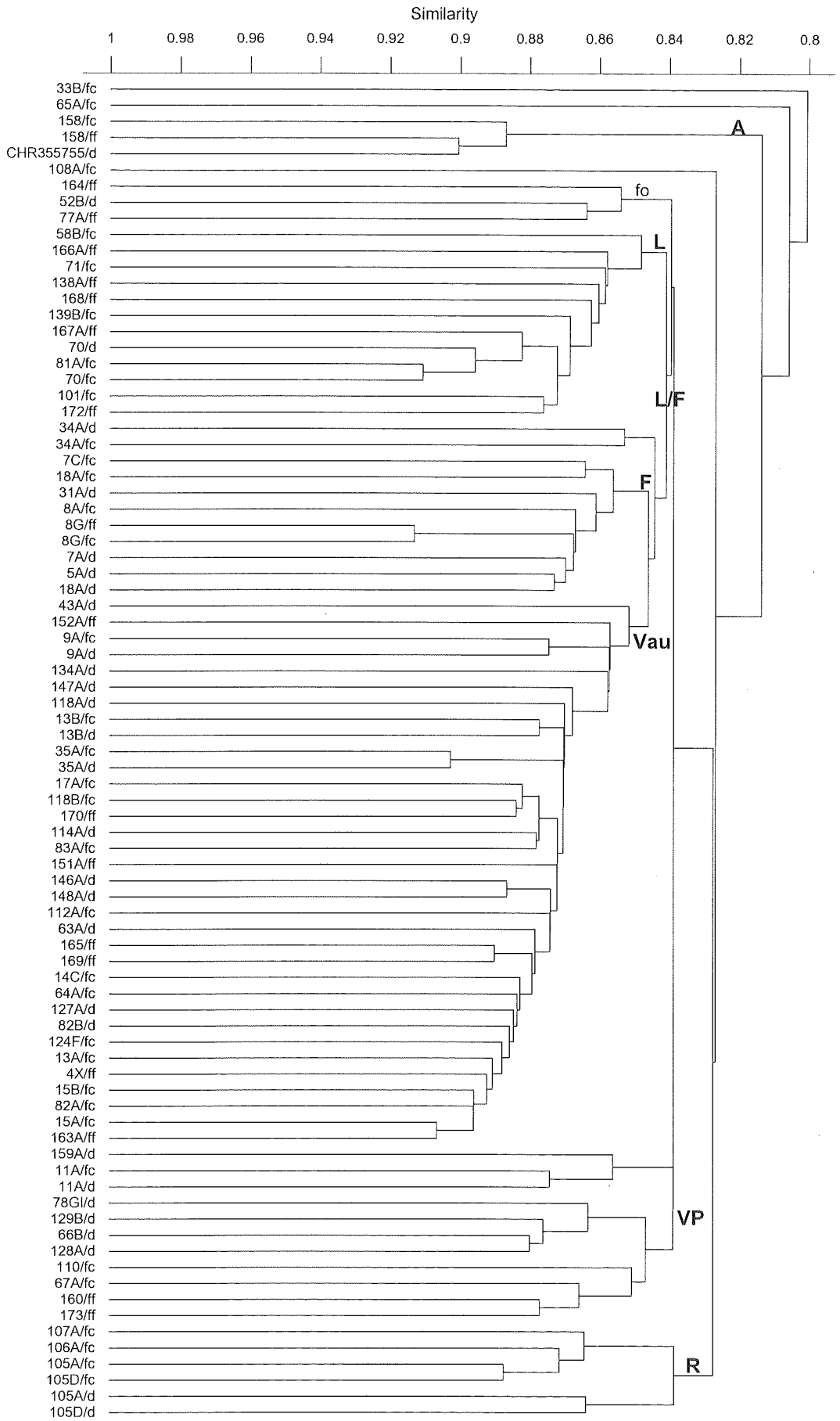


Fig. 2.19 Single linkage phenogram based on Data Set 3.

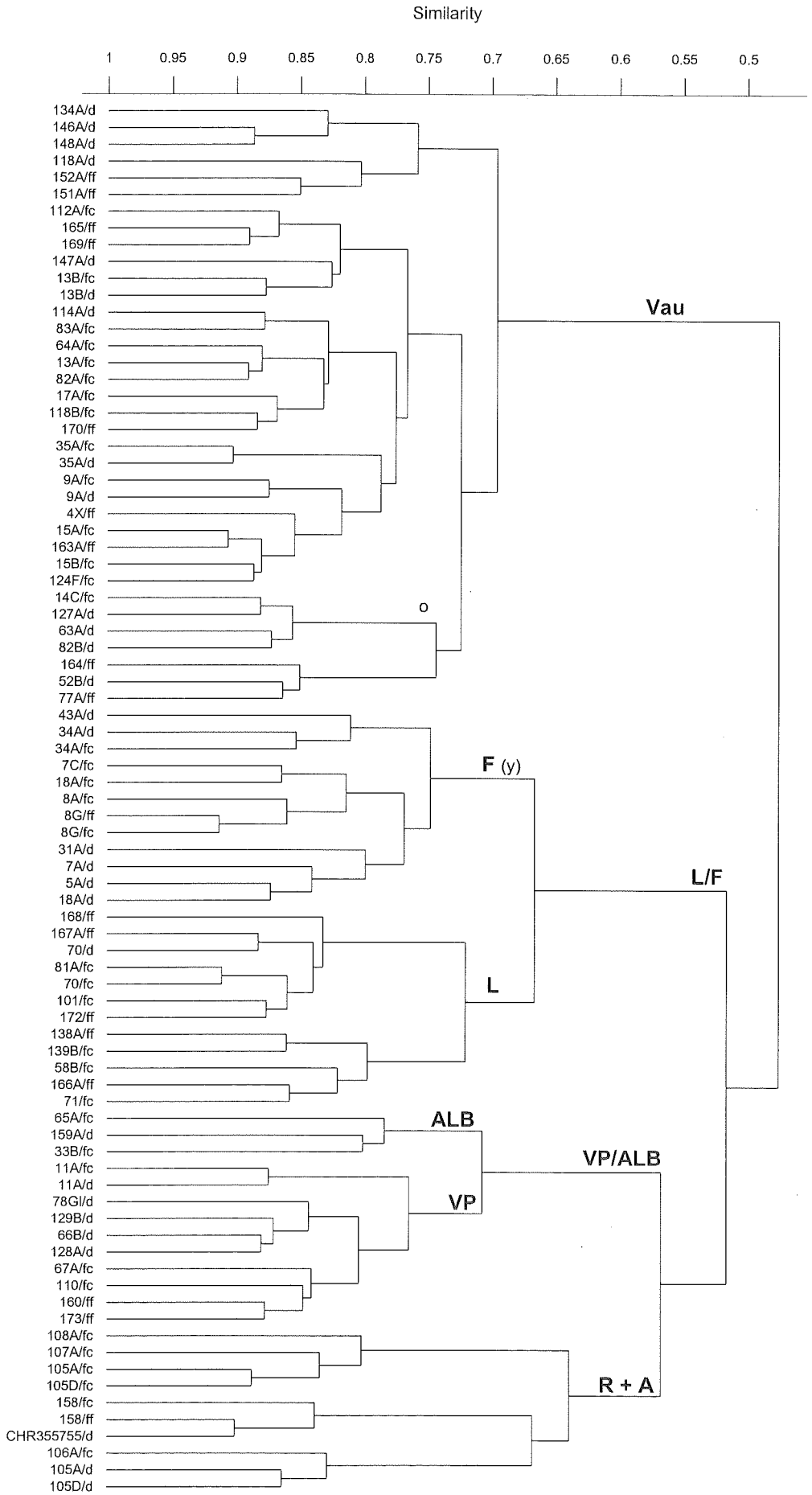


Fig. 2.20 Complete linkage phenogram based on Data Set 3.

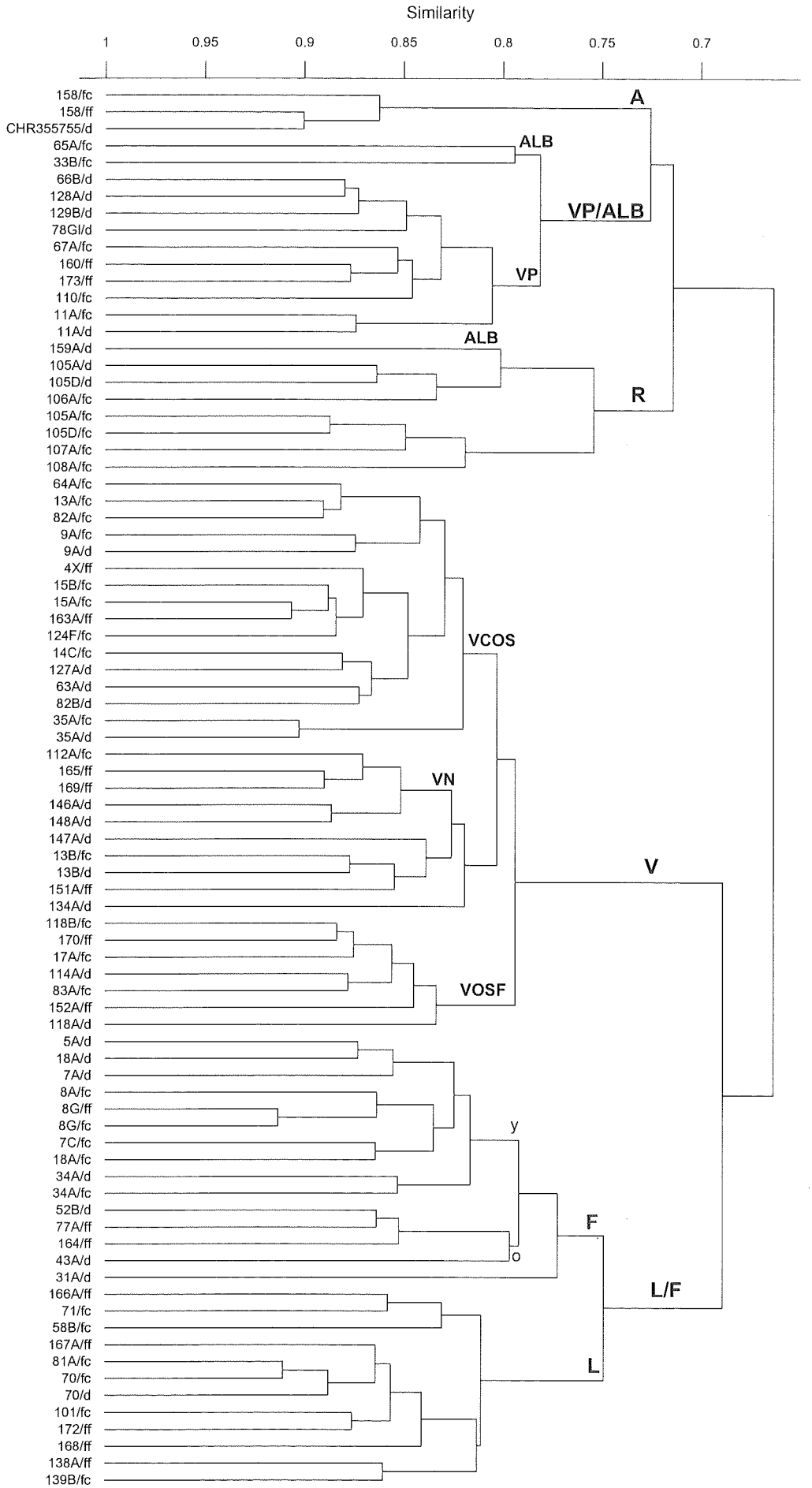


Fig. 2.21 WPGMA phenogram based on Data Set 3.

2.3.3.2 Numerical analyses of the reduced data set

Numerical analyses of Data Set 4:

Data Set 4 contained 33 OTUs and 80 morphological characters (Table 2.5), including 23 OTUs assessed for micromorphology (these OTUs were also included in the molecular studies).

Clustering

Cluster analysis performed on Data Set 4 resulted in four phenograms with the following overall cophenetic correlation coefficients (Table 2.9).

Table 2.9 Cophenetic correlations of the phenograms based on Data Set 4 with the similarity matrix from which they are derived.

	Overall Cophenetic Correlation Coefficient	
	Pearson	Spearman Rank
Average Linkage (UPGMA) Phenogram	0.75499	0.72873
Single Linkage Phenogram	0.71471	0.6889
Complete Linkage Phenogram	0.74621	0.72812
Weighted (WPGMA) Phenogram	0.68951	0.61431

UPGMA gave a slightly higher overall cophenetic correlation coefficient than the other three clustering methods. The UPGMA phenogram (Fig. 2.22) was therefore chosen as the basis for a detailed description of the overall arrangement of clusters.

Four main clusters could be distinguished in the UPGMA phenogram. Cluster designations are as follows V = 'Vauvilliersii', L/F = 'Leptophylla/Fulvida', R = 'Retorta' and A+ALB = 'Amoena/Albida' (as for previously described phenograms, Section 2.3.3.1). Cluster V and L/F linked together with a coefficient of 0.692 and R and A+ALB linked at 0.668. Clusters V, L/F, R and A+ALB linked at 0.633.

Cluster V was split into two clusters (VP and Vau) which joined at 0.748. Cluster VP contained specimens formerly described as *Cassinia vauvilliersii* var. *pallida* (66B/d, 67A/fc, and 11A/fc). Cluster Vau was composed of several smaller clusters: OTU 112A/fc, 148A/d and 134A/d formed a cluster, with the closest pairing being between the two North Island specimens 112A/fc (Mt. Egmont) and 148A/d (Pouaki Range) at 0.856. These last two OTUs were joined at 0.804 by OTU 134A/d from Danseys Pass/Otago. Other OTUs of

the cluster Vau were linked together at 0.797. Within this cluster, OTU 118B/fc and OTU 17A/fc, (both representatives of the 'Vauvilliersii S-Otago/Southland/Fiordland' group), formed a close pair (at 0.864). This pair was linked at 0.827 with another pair formed by a Mt. Cook specimen (OTU 64A/fc) and one from Otira Valley (OTU 13A/fc). Another specimen from the Otira Valley (OTU 13B/fc) formed a pair with OTU 35A/fc collected at Mt. Peel, Canterbury (0.849). This pair was joined by OTU 9A/fc, a specimen from the Garibaldi Ridge at 0.817.

The L/F cluster was composed of lesser clusters L and F, which were joined at 0.707. Cluster L was composed of one South Island (OTU 139B/fc) and 2 North Island (OTU 58B/fc and OTU 70/fc) specimens from the 'Leptophylla' group. OTU 139B/fc and OTU 70/fc were linked together at 0.844, while OTU 58B/fc joined more distantly (at 0.786). Cluster F primarily consisted of OTUs from the 'Fulvida' group, but also contained two specimens of the 'Vauvilliersii Cant./Otago/N-Southland' group: OTU 4X/ff and OTU 15A/fc that linked together at 0.849. This pair of OTUs specifically clustered with representatives of the 'Fulvida Central Otago/Inland Canterbury' group: 34A/fc (at 0.805) and 43A/d (at 0.795), which linked with a cluster containing representatives of the 'Fulvida Canterbury Coast' group (5A/d, 8G/fc, 7C/fc) (0.77). OTU 77A/ff a representative of the 'Fulvida Canterbury Coast' group with conspicuous orange exudates was an outlier of this 'Fulvida' cluster and joined at 0.754.

Cluster R was formed by four representatives of the 'Retorta' group. This cluster contained two representatives of the same plant, i.e., a dried field sample, not scored for floral character states (105A/d) and a specimen derived from cultivated material and assessed in a fresh state (105A/fc). OTU 105A/fc and OTU 106A/fc joined at 0.856 before joining at 0.812 with105A/d. OTU 108A/fc was linked to these three OTUs at 0.762. OTU 105A/d and OTU 105A/fc were the only genotypically identical pair carried through all previous steps of OTU number reduction.

Representatives of the former '*Cassinia vauvilliersii*' varieties '*albida*' and '*canescens*' (OTU 65A/f, OTU 159A/d, OTU 33B/fc) formed the ALB cluster and linked together with the representatives of 'Amoena' at a low level of similarity (0.723).

A vertical line might be drawn at the level of similarity at which here recognised groups begin to break up (0.735). At this level six clusters occurred: V = 'Vauvilliersii',

L = 'Leptophylla', F= 'Fulvida', R = 'Retorta', A = 'Amoena', and ALB = 'Albida'. These clusters were all present down to 0.72 similarity, where clusters A and ALB joined together.

The cophenetic correlation coefficient for the UPGMA phenogram (Fig. 2.26) dropped to 0.925 when the OTU pair 118B/fc and 17A/fc (both from the 'Vauvilliersii S-Otago/Southland/Fiordland' group) linked with another 'Vauvilliersii' pair: OTU 64A/fc and OTU 13A/fc at 0.827. The correlation coefficient then stabilised between 0.92 and 0.95 for the links occurring between 0.819 and 0.804. It dropped subsequently to 0.84 at 0.797 where all the OTUs of the cluster Vau linked together. A further drop down to 0.82 occurred at 0.77, where the coastal 'Fulvida' representatives (5A/d, 8G/fc, 7C/fc) joined the representatives of the 'Fulvida Central Otago/Inland Canterbury' group (43A/d, 34A/fc, 4X/ff, 15A/fc) and when all the representatives of the Vau cluster linked together at 0.770 the correlation coefficient was down to 0.76. From the lowest value of 0.735, when OTU 108A/fc joined the 'Retorta' cluster at 0.762 the coefficient rose slightly up to 0.825 for the links occurring between 0.759 and 0.707. The correlation coefficient dropped finally down to 0.755 at 0.692, where the V cluster joined the L/F cluster, and stabilised around 0.75.

The cophenetic correlation plot (Fig. 2.26) showed that the correlation between the similarity matrix and the phenogram was always relatively high (between 0.762 and 1.0), with no dramatic drop of the correlation coefficient occurring. It stabilised at a relatively high value of about 0.75.

The fact that the overall cophenetic correlation coefficient of the UPGMA phenogram was just slightly higher than that of the phenograms created using single linkage, complete linkage and WPGMA clustering methods (Table 2.9) made it necessary to describe these three phenograms briefly (Figs. 2.23 -2.25).

WPGMA clustering (Fig. 2.25) gave almost the same pattern as seen in the UPGMA phenogram. The six main clusters (A, ALB, V, L, F, and R) were present at a similarity level > 0.72. The only difference between the two phenograms was that in the WPGMA phenogram the A+ALB cluster was linked to the V cluster and not to the R cluster as in the UPGMA phenogram. The R cluster was the last to join, at 0.64, in the WPGMA phenogram. The cophenetic correlation coefficient was only 0.69 for this step.

The link between A+ALB and R in the UPGMA phenogram had a cophenetic correlation coefficient of 0.75. The joining of the A+ALB cluster with the V cluster at 0.677 in the WPGMA phenogram was characterised with a relatively higher cophenetic correlation coefficient of 0.82 (Fig. 2.29), while the cophenetic correlation coefficient for the link of V and F/L in the UPGMA phenogram was only 0.75 (Fig. 2.26).

The complete linkage phenogram (Fig. 2.24) also showed a very similar cluster arrangement compared with that resulting from UPGMA analysis. OTU 77A/ff, a coastal representative of the 'Fulvida' group with conspicuous orange exudates, was the last to link with the F cluster in the UPGMA phenogram, grouping together with the other coastal representatives of the F cluster in the complete linkage phenogram. The overall cophenetic correlation coefficient for the joining of OTU 77A/ff varied less than 0.03 between the two clustering methods. The main clusters V, L, F, R, A, and ALB are present but joined at a slightly different levels of similarity. A line drawn at >0.665 to separate the clusters A and ALB was very close to the similarity level at which the clusters Vau and VP were linked together (at 0.676).

Single linkage clustering (Fig. 2.23) resulted in two main clusters linked at 0.760. One of these clusters contained R and A as a subcluster with OTU 159/A from the cluster ALB joining the cluster R at 0.800. OTU 33B/fc joined this main cluster last at 0.773. The other main cluster was formed by several smaller clusters, all of them already known from previous cluster analyses. Cluster F in the single linkage phenogram contained only coastal representatives of the 'Fulvida' group with OTU 7C/fc linked with OTU 8G/fc at 0.835 and OTU 5A/d joining the pair at 0.839. OTU 43A/d, a representative of the 'Fulvida Central Otago/Inland Canterbury' group and OTU 77A/ff ('Fulvida Canterbury Coast' group) were quite isolated. OTU 34A/d, the other representative of the 'Fulvida Central Otago/Inland Canterbury group' was linked to cluster V with the OTUs 4X/ff and 15A/fc, all of them members of cluster F in the other phenograms. When the pair 4X/ff and 15A/fc linked with a cluster containing 118B/fc and 17A/fc, from the 'Vauvilliersii S-Otago/Southland/ Fiordland' group, and 64A/fc and 13A/fc 'Vauvilliersii' from Mt. Cook and Otira Valley the cophenetic correlation coefficient dropped as low as 0.7 (Fig. 2.27). The OTUs 65A/fc and 66B/d were outliers of the second main cluster.

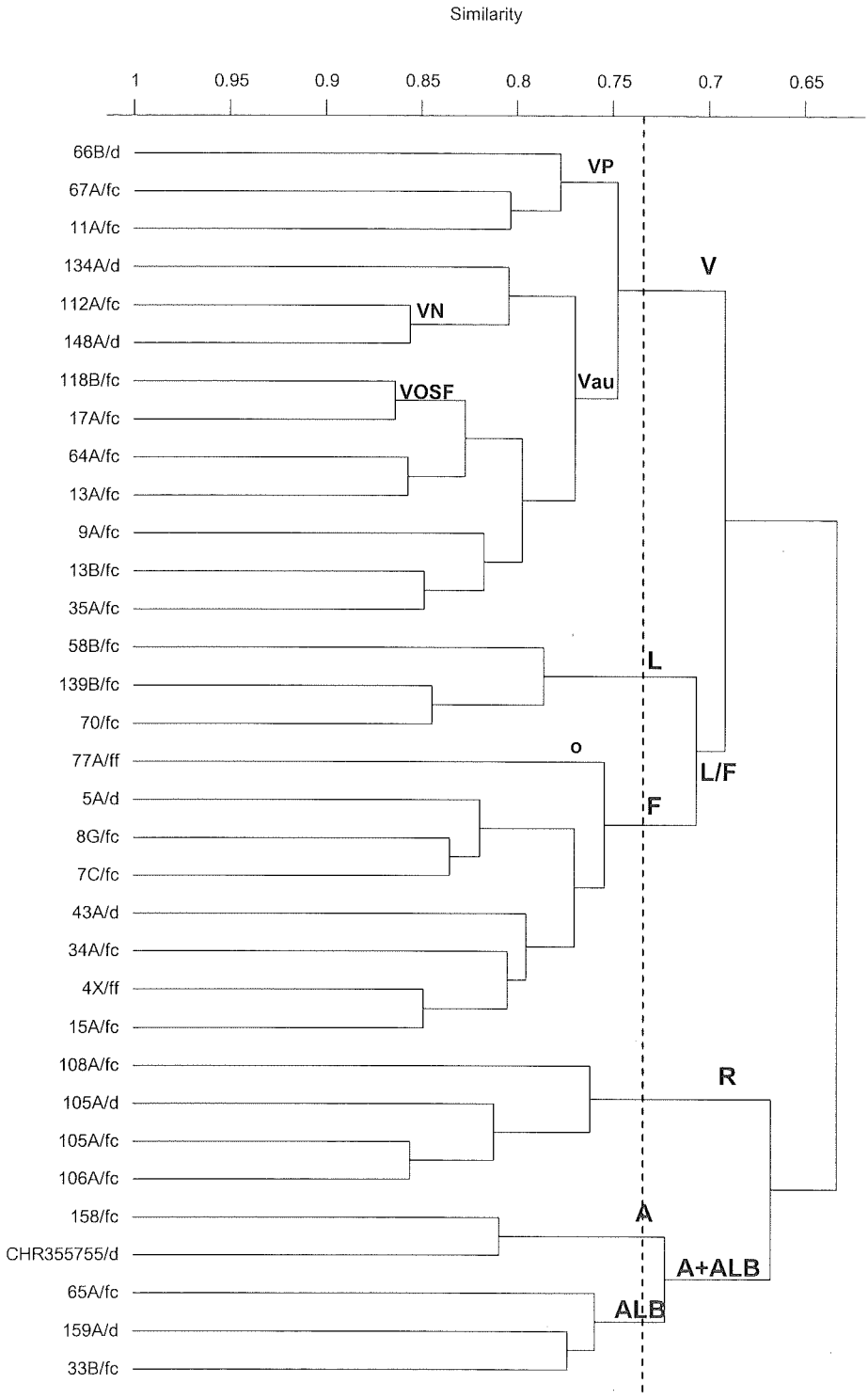


Fig. 2.22 UPGMA phenogram based on Data Set 4.

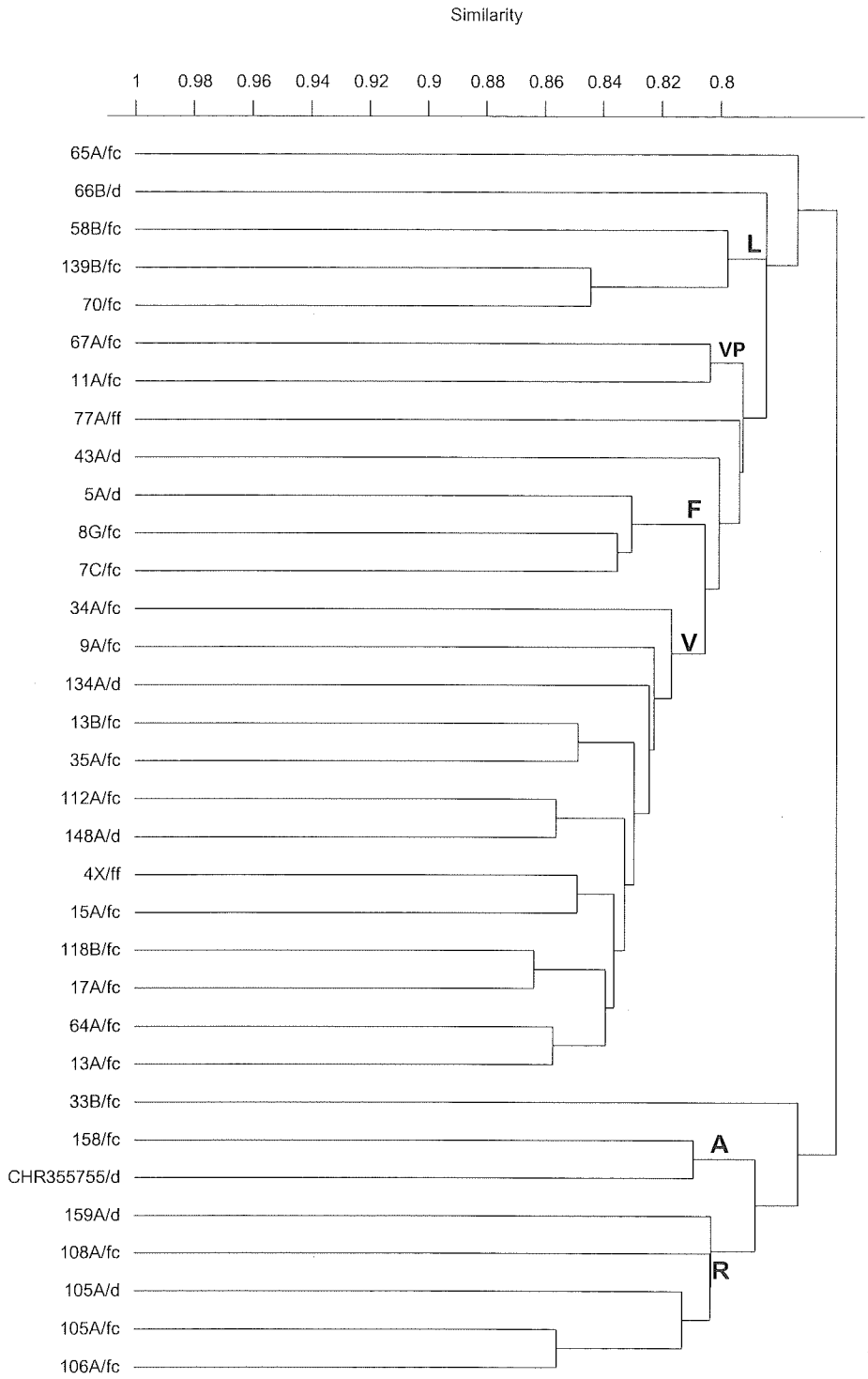


Fig. 2.23 Single linkage phenogram based on Data Set 4.

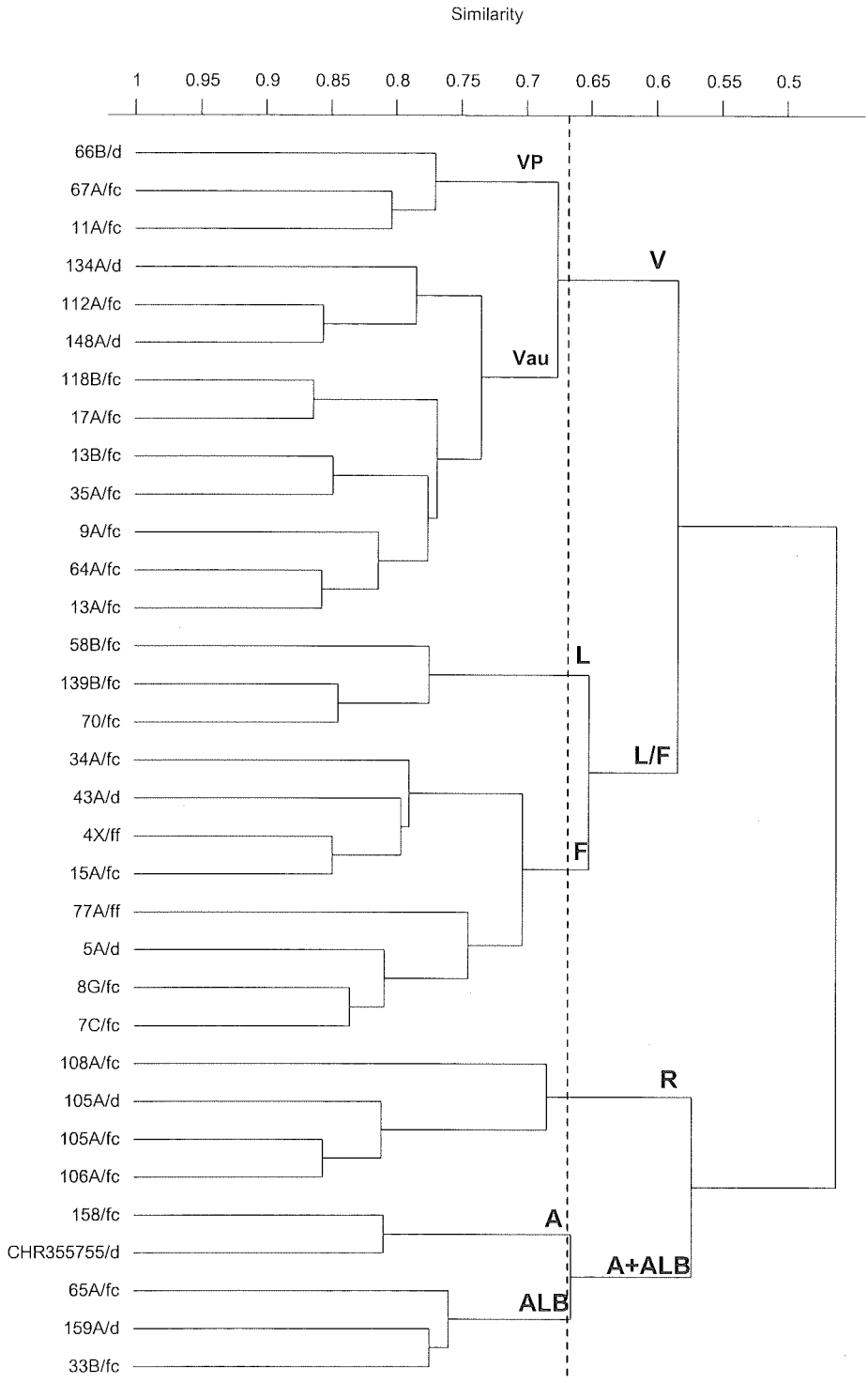


Fig. 2.24 Complete linkage phenogram based on Data Set 4.

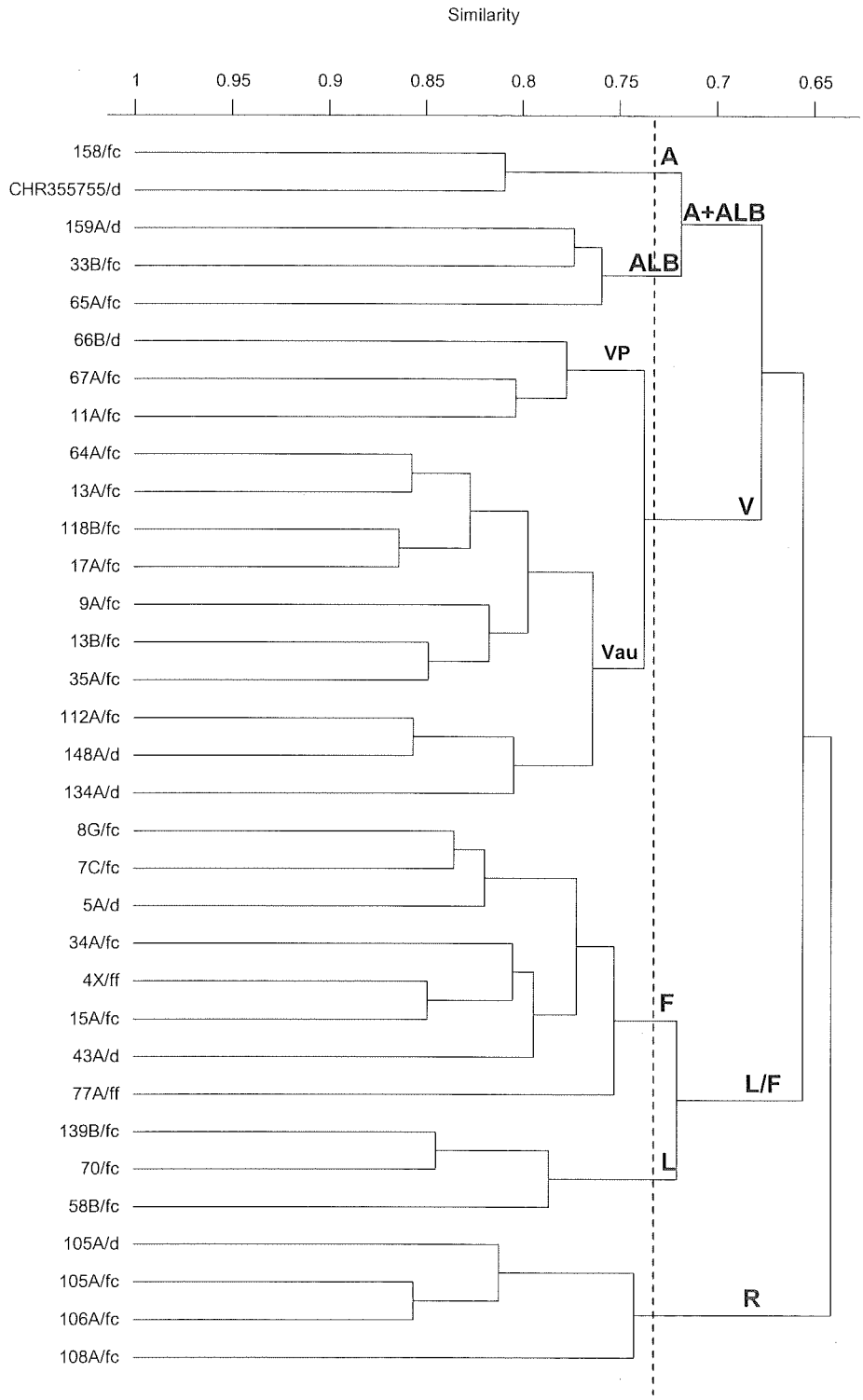


Fig. 2.25 WPGMA phenogram based on Data Set 4.

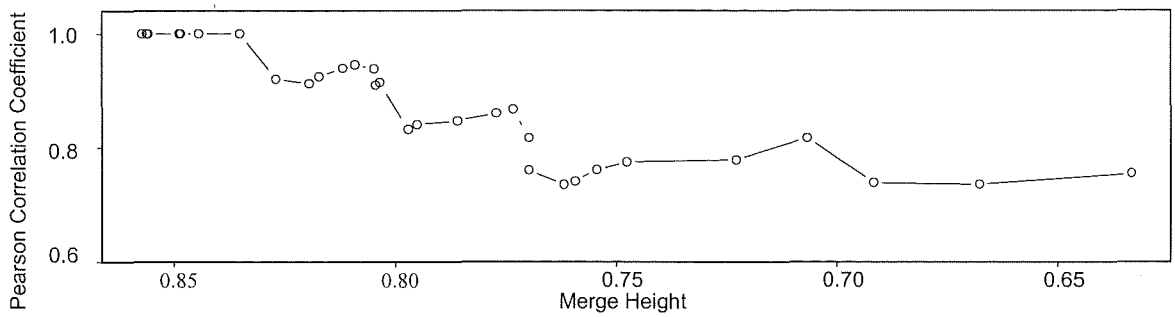


Fig. 2.26 Plot showing the change in the cophenetic correlation coefficient as taxa are clustered in the UPGMA phenogram based on Data Set 4.

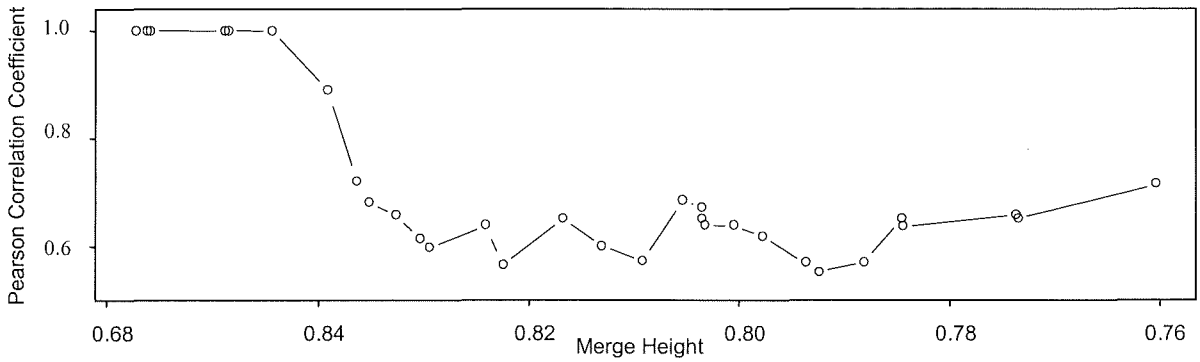


Fig. 2.27 Plot showing the change in the cophenetic correlation coefficient as taxa are clustered in the single linkage phenogram based on Data Set 4.

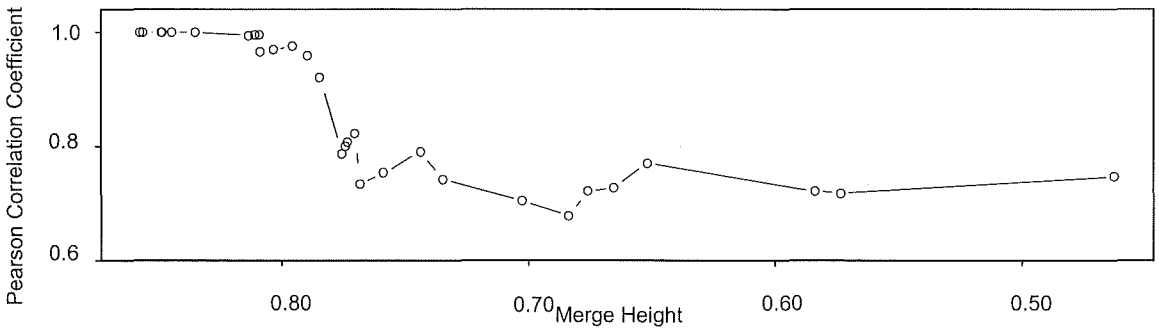


Fig. 2.28 Plot showing the change in the cophenetic correlation coefficient as taxa are clustered in the complete linkage phenogram based on Data Set 4.

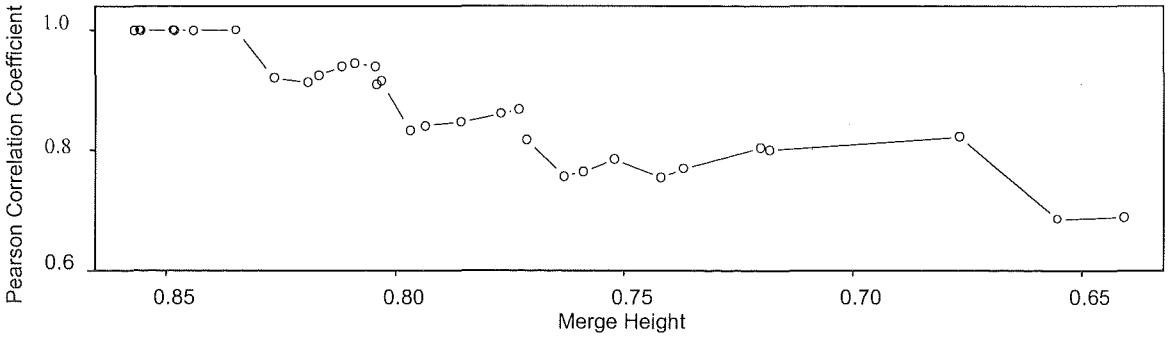


Fig. 2.29 Plot showing the change in the cophenetic correlation coefficient as taxa are clustered in the WPGMA phenogram based on Data Set 4.

Comparison of the UPGMA phenograms based on Data Set 3 and 4

The overall structure or the two UPGMA phenograms generated from analysis of Data Sets 3 and 4 (Fig. 2.18 and Fig. 2.22) was similar. Due to the reduction of OTUs, smaller subclusters, such as VOSF, VN, and o were less obvious in the UPGMA phenogram based on Data Set 4, e.g., cluster o was only represented by OTU 77A/ff in the phenogram based on Data Set 4. The representatives of the VCOS/VWC cluster in the UPGMA phenogram based on Data Set 3 did not longer form a cluster in the UPGMA phenogram based on Data Set 4.

Two OTUs changed clusters: OTU 4X/ff and 15A/fc, together forming a subcluster with 124F/fc, 15B/fc, and 163A/ff, within the VCOS cluster, one of the three subclusters of the cluster Vau of the UPGMA phenogram based on Data Set 3, appeared in the phenogram based on the reduced Data Set 4 in the y subcluster of cluster F. Cluster F in the phenogram based on Data Set 4 was divided into two subclusters. One of them contained coastal representatives of the 'Fulvida' group (5A/d, 8G/fc, 7C/fc), the other the representatives of the 'Fulvida Central Otago/Inland Canterbury' group (43A/fc, 34A/d) and the two OTUs 4X/ff and 15A/fc originally assigned to the 'Vauvilliersii Cant./Otago/ N-Southland' group (Section 2.3.1) and placed within the cluster VCOS in the previous analyses of Data Sets 1-3. The cluster VP/ALB separated at a relatively low level of similarity (0.774) into two clusters (VP and ALB) in the UPGMA phenogram based on Data Set 3. In the UPGMA phenogram based on Data Set 4 the ALB cluster formed a distinct cluster which joined the A cluster at 0.723. The R cluster, formerly (in the UPGMA analysis of Data Set 3) linked to the A cluster at 0.714 joined the A+ALB cluster at 0.67 in the UPGMA phenogram based on Data Set 4.

Ordination

The six clusters V, L, F, R, A, and ALB linked to the level of 0.735 in the UPGMA phenogram based on Data Set 4 were also reflected in the PCO plots. The first three axes of the PCO explained nearly 40% of the variation in the data set, whith each axis individually explaining 18.2%, 12,6% and 8.6% respectively. The graphs (Fig. 2.30 - Fig. 2.32) highlighted the grouping of OTUs which reflected clusters from the UPGMA phenogram (above a similarity of 0.723), but also identified outliers and illustrated the overlap between clusters.

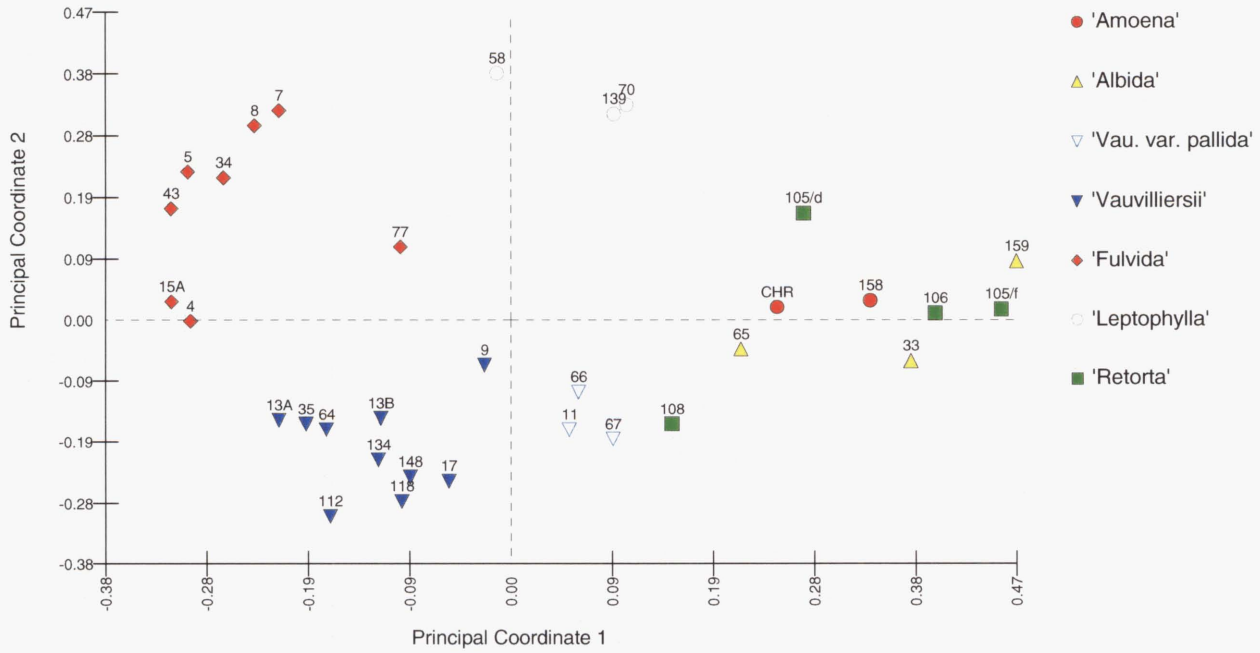


Fig. 2.30 Plot of principal coordinate analysis 1 vs. 2 generated from Gower's General Coefficient of Similarity based on Data Set 4.

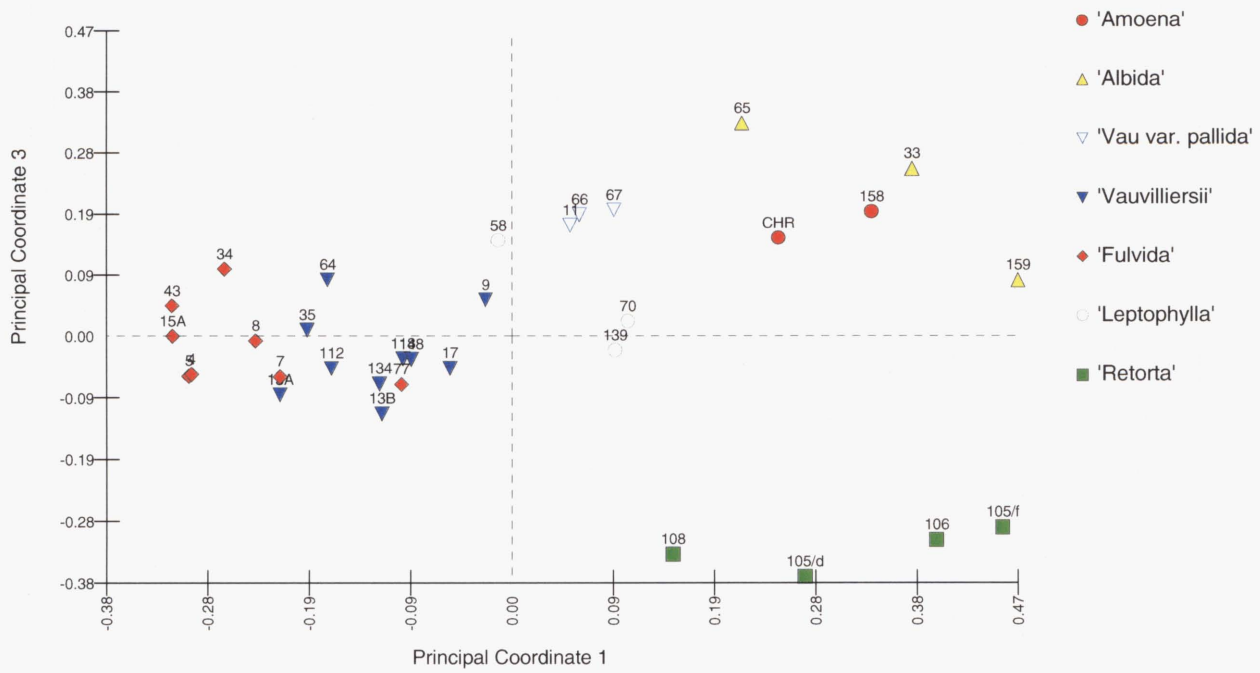


Fig. 2.31 Plot of principal coordinate analysis 1 vs. 3 generated from Gower's General Coefficient of Similarity based on Data Set 4.

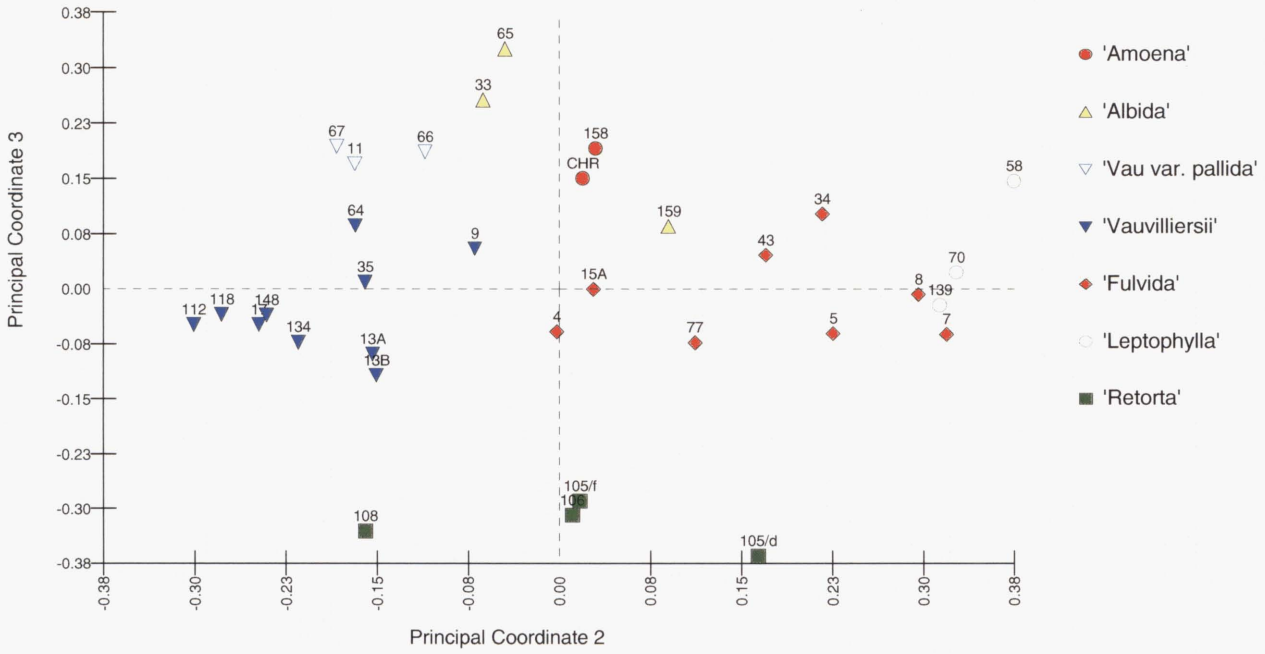


Fig. 2.32 Plot of principal coordinate analysis 1 vs. 3 generated from Gower's General Coefficient of Similarity based on Data Set 4.

The 'Vauvilliersii' and the 'Fulvida' groups were positioned to the left of all other groups on principal coordinate 1. The 'Vauvilliersii var. pallidus' group and the 'Leptophylla' group were both centred around the origin, while the 'Amoena', 'Albida' and 'Retorta' groups fell to the right of these based on principal coordinate 1. Principal coordinate 2 separated the 'Vauvilliersii' group from the 'Fulvida' group and distinguished the 'Leptophylla' and the 'Vauvilliersii var. pallidus' groups from each other. OTU 15A/fc and OTU 4X/ff were located in between the 'Vauvilliersii' group and the 'Fulvida' group based on principal coordinate 2 (Fig. 2.30, Fig. 2.32). These OTUs were also present in the 'Fulvida' cluster (F) in the phenogram based on Data Set 4, but placed in the 'Vauvilliersii' cluster (V) in the phenograms based on the less reduced data sets. Principal coordinate 3 clearly identified outliers, separating 1) the 'Retorta' group from other OTUs; 2) the 'Albida' group, although with some affinity to the 'Amoena' group; and 3) the three OTUs (11A/fc, 66B/d, and 67A/fc), which had also been found to group together in the subcluster VP of the 'Vauvilliersii' cluster in the UPGMA phenogram based on Data Set 4 (Fig. 2.31 and Fig. 2.32).

Box-Plots of character ranges within groups

Univariate statistics for all characters included in the numerical analysis were computed and presented in the form of box-plots (Fig. 2.33). The box-plots show character distributions for the quantitative and ordered qualitative characters within each of the 6 groups distinguished in the UPGMA phenogram based on analysis of Data Set 4. The boxes show interquartile range (25% and 75% quartiles (upper and lower limits of each box)) and median (central line in each box). Whiskers indicate smallest and largest observations within 1.5 times the interquartile range from quartiles. The outliers, indicated by lines outside of the whiskers, are points greater than 1.5 times from quartiles.

Twelve of the 79 characters showed total ranges that either overlapped or were identical for all groups, these being: branchlet diameter (4), colour gradient of adaxial surface of young leaf (11), colour depth of adaxial surface of young leaf (13), secondary leaf shape (21), secondary apex (23), exudate density on adaxial surface of the lamina of old leaf (41), leaf spacing (47), compactness of panicle (48), tips of outer involucral bracts spreading (69), tips of inner involucral bracts spreading (75), ratio of receptacle scale length to width (82), and density of twin hairs on achene (89).

All other characters differentiated at least two groups from each other. Several characters had different ranges for two, three, or even four groups (e.g. capitulum length (55), involucre length (56), and involucre width (57)). A single character was not found displaying totally different ranges for five or all of the six groups.

Several characters were found to be invariant in at least one cluster, e.g., habit and growth form (1) in group 1, 3, 5, and 6; main leaf shape (20) in group 4, 5 and 6.

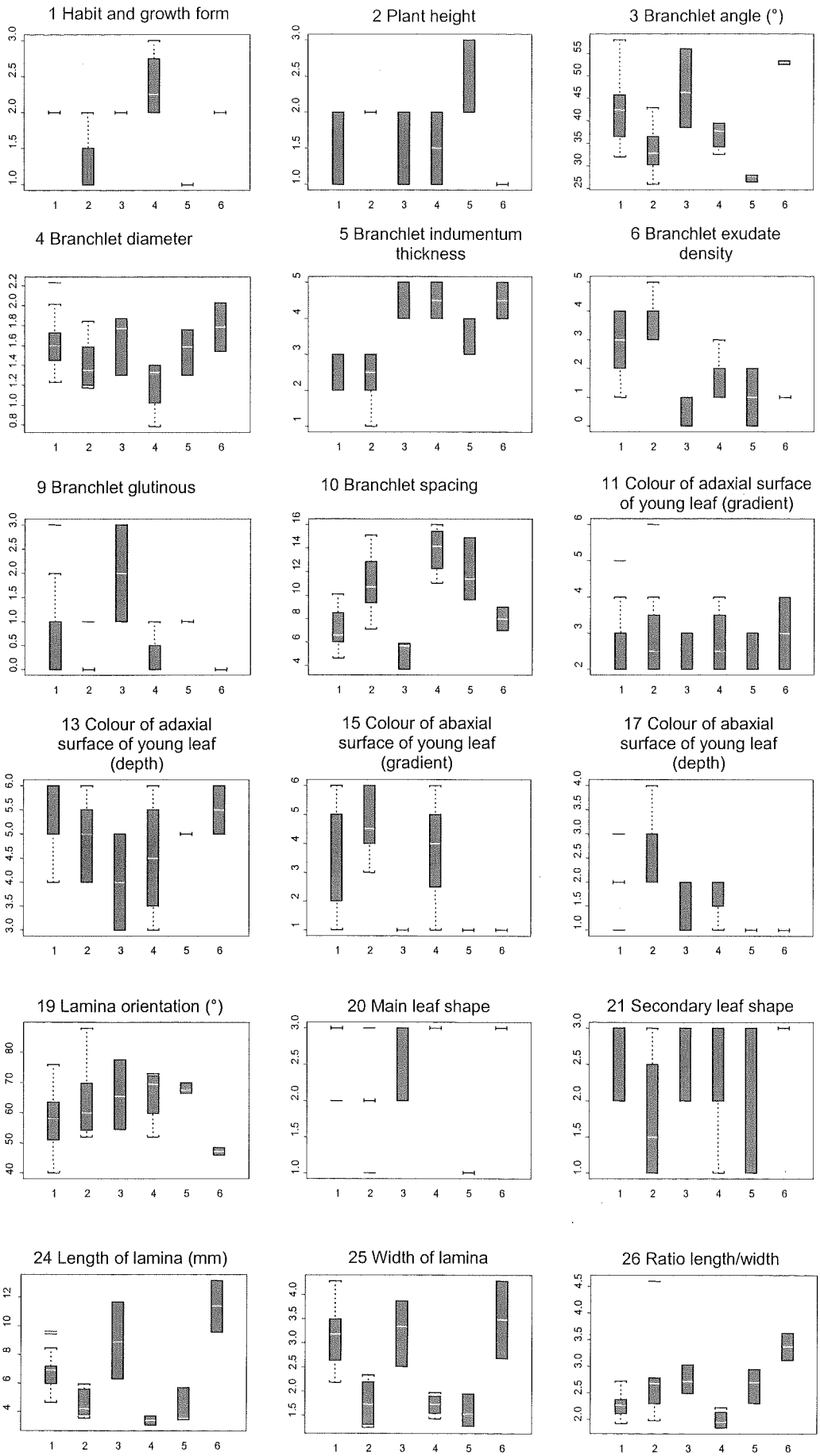


Fig. 2.33 see caption on page 136

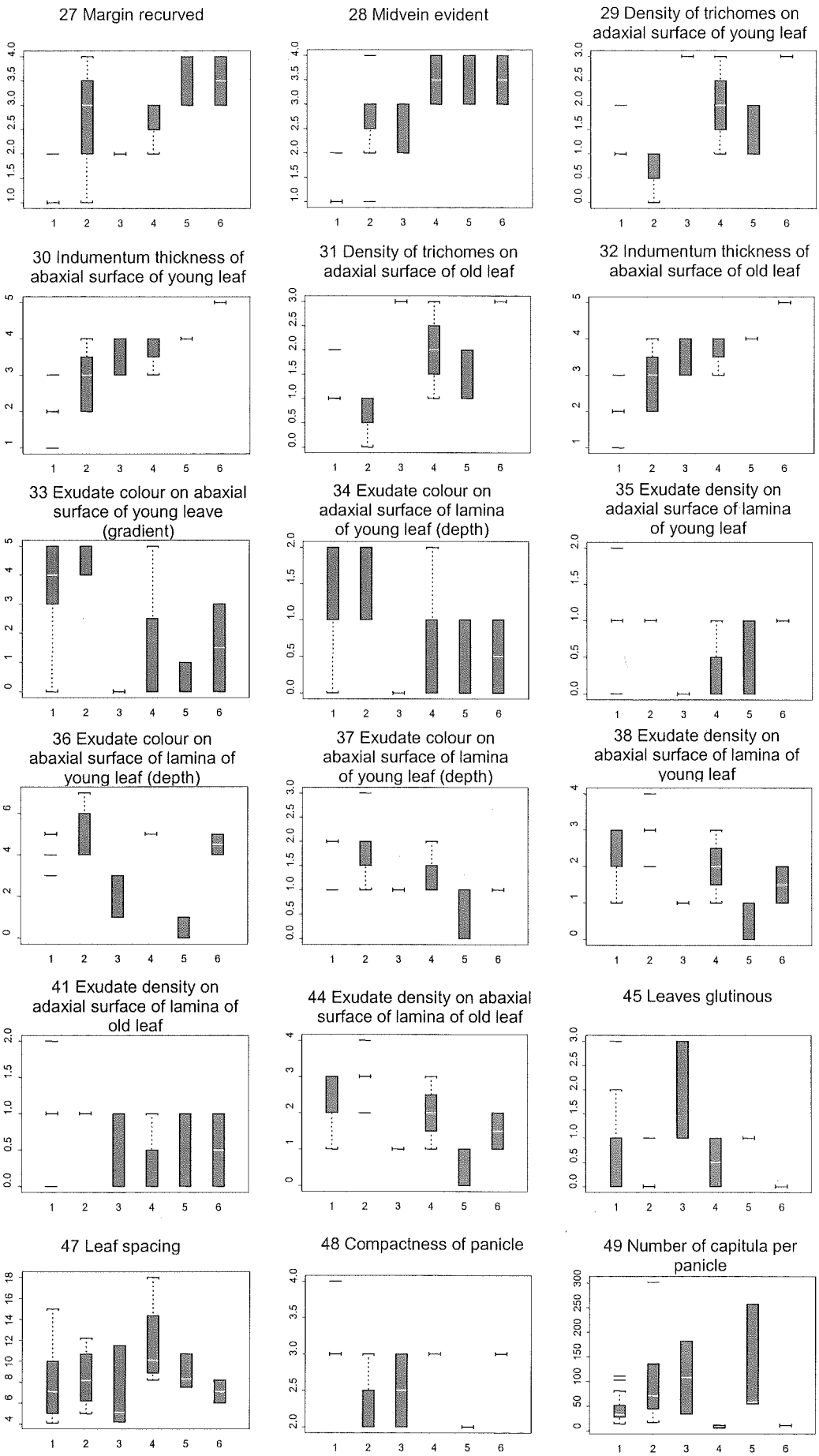


Fig. 2.33 see caption on page 136

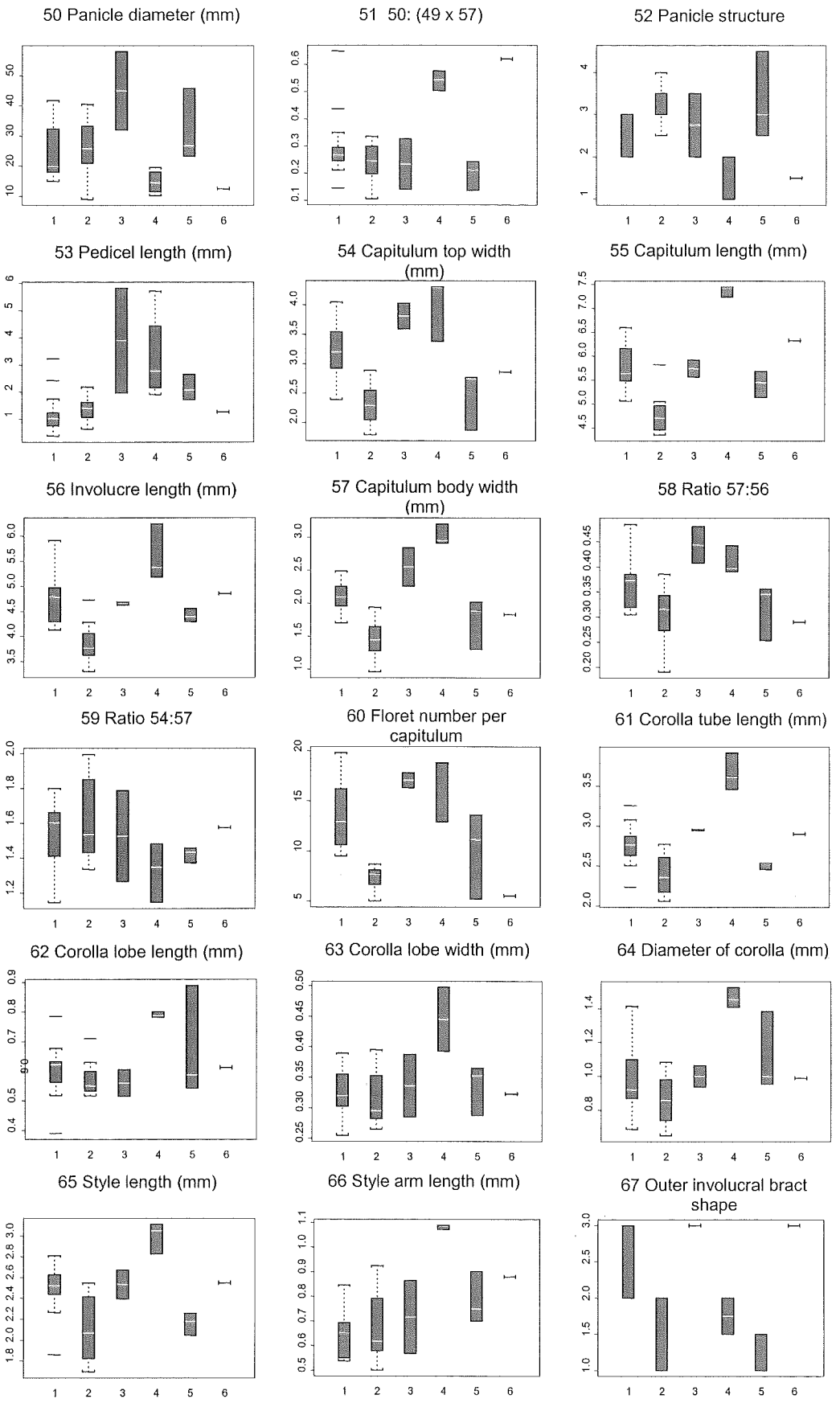


Fig. 2.33 see caption on page 136

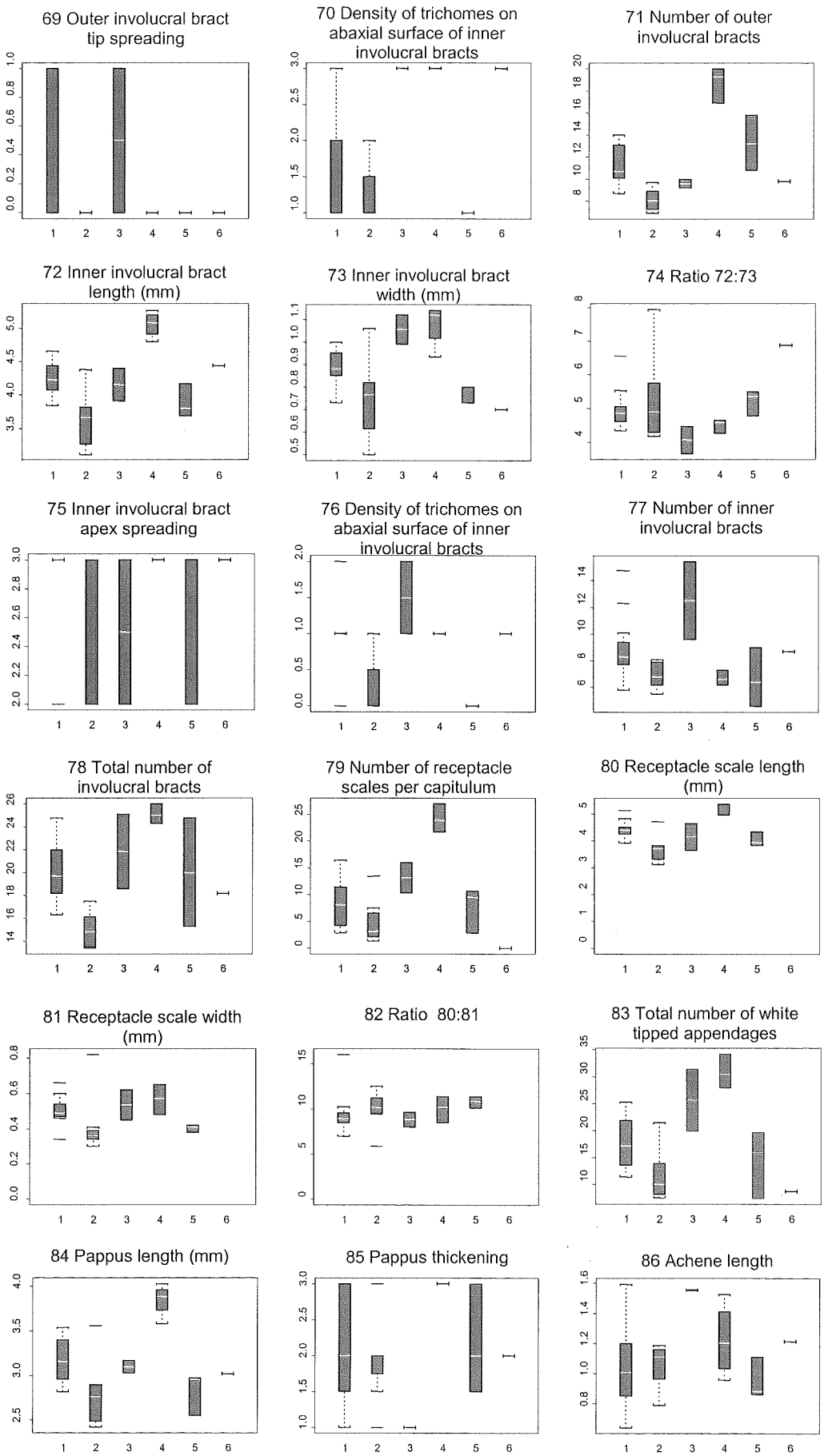


Fig. 2.33 see caption on page 136

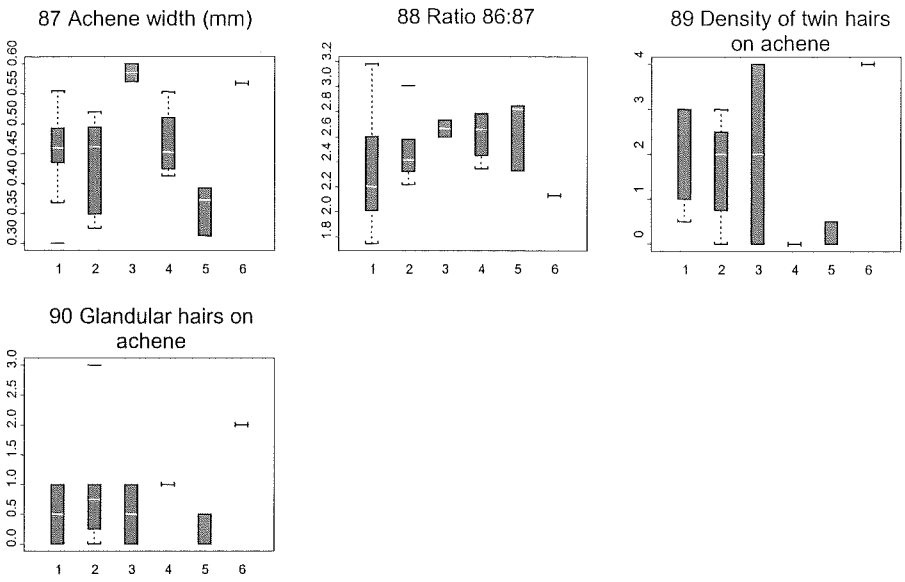


Fig. 2.33 Box-plots showing character distributions within each of the 6 groups distinguished in the UPGMA phenogram based on analysis of Data Set 4: 1 = 'Vauvilliersii' group, 2 = 'Fulvida' group, 3 = 'Albida' group, 4 = 'Retorta' group, 5 = 'Leptophylla' group, 6 = 'Amoena' group. Median, 25 and 75 percentiles, whiskers maximum point with >1.5x interquartile range, and outliers are represented.

2.3.3.3 Numerical analysis of reduced data sets with additional microcharacters and of a character subset containing microcharacters only

UPGMA analysis was based on the reduced data set (Data Set 4) with 33 OTUs and 11 additional microcharacters for 23 OTUs (Data Set 5) (Fig. 2.34). The overall cophenetic coefficient values for this phenogram were 0.74516 (Pearson) and 0.71631 (Spearman Rank). The topology of this phenogram was nearly identically to that based on Data Set 4, which excluded microcharacters. Only OTU 159, formerly placed in the ALB cluster moved into the R cluster. The similarity level above which all the clusters (V, L, F, A, R, and ALB) are present was 0.717, where cluster L and F were joined together. This was also reflected in the UPGMA phenogram based on Data Set 4, where these OTUs linked at or above the similarity level of 0.72.

The UPGMA analysis of Data Set 6, containing the 23 OTUs for which microcharacters were available only, resulted in a similar phenogram (Fig. 2.35) to those based on Data Set 4 and 5. The overall cophenetic coefficient was slightly higher compared with that for the UPGMA phenogram based on Data Set 5 (0.80185 (Pearson) and 0.76596 (Spearman Rank)). All the clusters (V, L, F, A, R, and ALB) were present above the similarity level of 0.72, where cluster L and cluster F joined. OTU 15A/fc moved back into the cluster V, where it was also placed in the UPGMA phenograms resulting from analyses of the Data Sets 1-3.

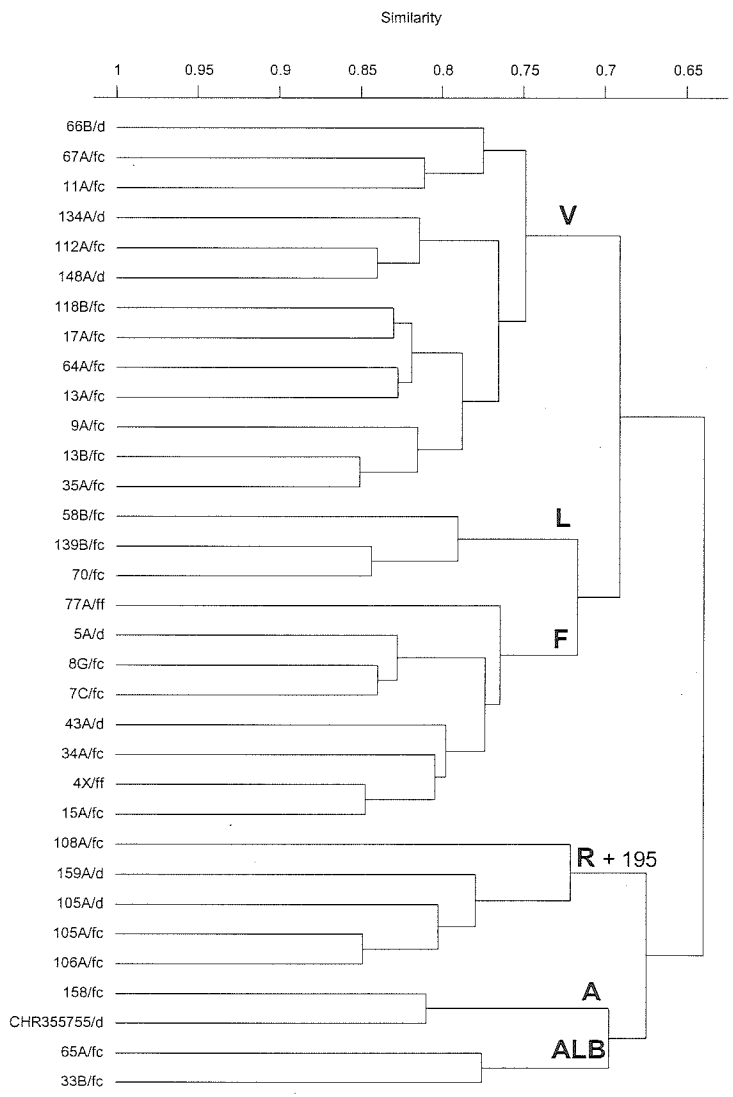


Fig. 2.34 UPGMA phenogram based on Data Set 5.

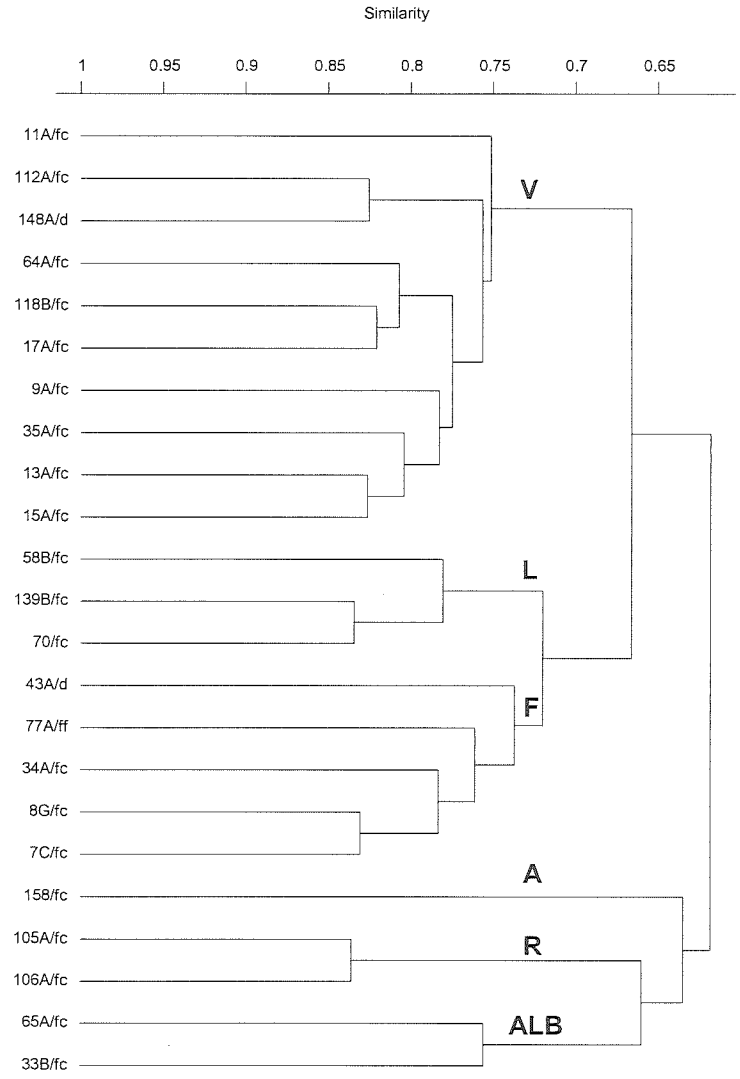


Fig. 2.35 UPGMA phenogram based on Data Set 6.

Microcharacter box-plots

A break down of microcharacter distributions for the UPGMA phenogram based on analysis of Data Set 6, (containing 75 macromorphological characters, plus 15 microcharacters) was shown in form of box-plots (Fig. 2.36) (refer to section 2.3.3.2).

As for the macrocharacters (Section 2.3.3.2), none of the microcharacters differentiated all six groups present in the phenogram. However, unlike the macrocharacters, total ranges were never identical or strongly overlapping for all groups based on any of the microcharacters. For all microcharacters, at least one group overlapped or covered with its range the range of one or several other clusters. For example, the density of the twin hairs on the achene (MC8), for which three totally different character ranges were present, i.e., group 4 ('Leptophylla') and group 5 ('Retorta') had identical ranges, but together were clearly differentiated from group 2 ('Vauvilliersii'). This may be compared with group 1 ('Fulvida') and group 3 ('Albida') for which the ranges for character MC8 overlapped with that for group 2, 4 and 5. Group 6 ('Amoena') was differentiated from all other groups for character MC8. This situation, of three distinct character ranges (not always for the same groups) was also the result for nine other characters: pappus width at the tip (MC1), width of apical pappus cells (MC3), pappus width at the widest part (MC4), pappus width at the centre (MC5), length of barbellae (MC6), distance between barbellae and axis (MC7), ratio of corolla tube length and the anther insertion point in corolla (MC12), anther connective base length (MC13), and ratio of anther connective base length and anther basal appendages length (MC15). The number of apical cells (MC2), the density of glandular hairs on achene (MC9), and the anther insertion point in the corolla (MC10) differentiated only one group (MC2 and MC9: 'Amoena'; MC10: 'Retorta') from all the other groups.

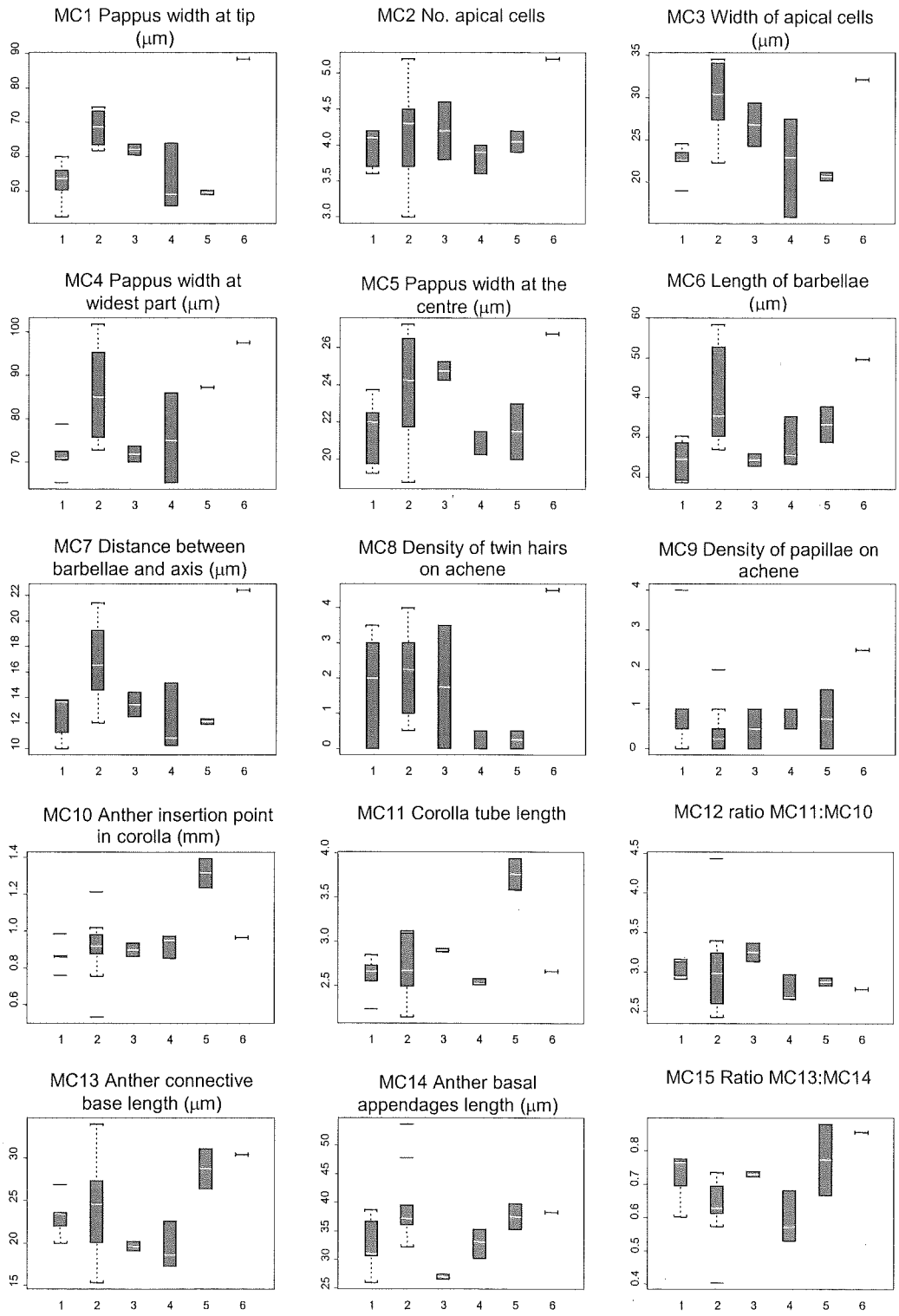


Fig. 2.36 Box-plots showing microcharacter distributions within each of the 6 groups distinguished in the UPGMA phenogram based on analysis of Data Set 6: 1 = 'Fulvida' group, 2 = 'Vauvilliersii' group, 3 = 'Albida' group, 4 = 'Leptophylla' group, 5 = 'Retorta' group, 6 = 'Amoena' group. Median, 25 and 75 percentiles, whiskers maximum point with >1.5x interquartile range, and outliers are represented.

UPGMA analysis on microcharacters only

Clustering based on 15 microcharacters only (Data Set 7) resulted in a phenogram with unique topology compared with the results of all previous analyses (Fig. 2.37). The overall cophenetic correlation coefficients were 0.81488 (Pearson) and 0.82521 (Spearman Rank). The cophenetic correlation coefficient graph is shown in Fig. 2.38. Three main clusters could be distinguished within this phenogram (based on Data Set 7): 1) including a cluster containing representatives of the 'Retorta' group plus OTU 7C/fc (previously been assigned to the 'Fulvida' group); 2) a cluster with representatives of the 'Vauvilliersii' group plus OTU 58B/fc (previously assigned to the 'Leptophylla' group) linked to 11A/fc (formerly grouped within the subcluster VP of the 'Vauvilliersii' cluster); and 3) a cluster containing representatives of F and L from previous analyses with the two representatives of cluster ALB (33B/fc and 65A/fc). OTUs 9A/fc and 17A/fc joined these three clusters at 0.723. The last cluster to join (at 0.61) consisted of OTU 64A/fc (from Mt. Cook), which was linked at 0.84 together with OTU 118B/fc (a representative of the southern 'Vauvilliersii' population), and joined with the representative of the 'Amoena' group (OTU 158/fc) at 0.71.

Some clusters present in the microcharacters based phenogram showed congruence with those resulting from previous analyses, especially the ones showing high levels of similarity and/or reflecting a good fit of the similarity matrix (indicated by a high cophenetic correlation coefficient, Fig. 2.38). OTU 77A/ff and OTU 34A/fc, which grouped together in cluster F in previous analyses, were shown to link together strongly (0.89) based on microcharacters. Similarly, another strongly linked pair (0.865) based on microcharacters was OTU 8G/fc and OTU 70/fc, both coastal, small leaved specimens.

OTU 11A/fc (a specimen from cluster V in previous analyses) and OTU 58B/fc (an OTU from cluster L in previous analyses) forming a pair (at 0.86) resulted in a first relatively big drop in the cophenetic correlation coefficient down to 0.85. The coefficient rose up to 0.9 when OTU 43A/d, a representative of the 'Fulvida' group, joined OTU 77A/ff and OTU 34A/fc, which themselves were linked at 0.85. Both representatives of cluster R in previous analyses (105A/fc and 106A/fc) were also linked together (at 0.826). When OTU 33B/fc joined the pair formed by OTU 8G/fc and OTU 70/fc, the cophenetic correlation coefficient dropped to 0.78. OTU 7C/fc linked to the R cluster at 0.751. At this point the cophenetic correlation coefficient reached a low of 0.575.

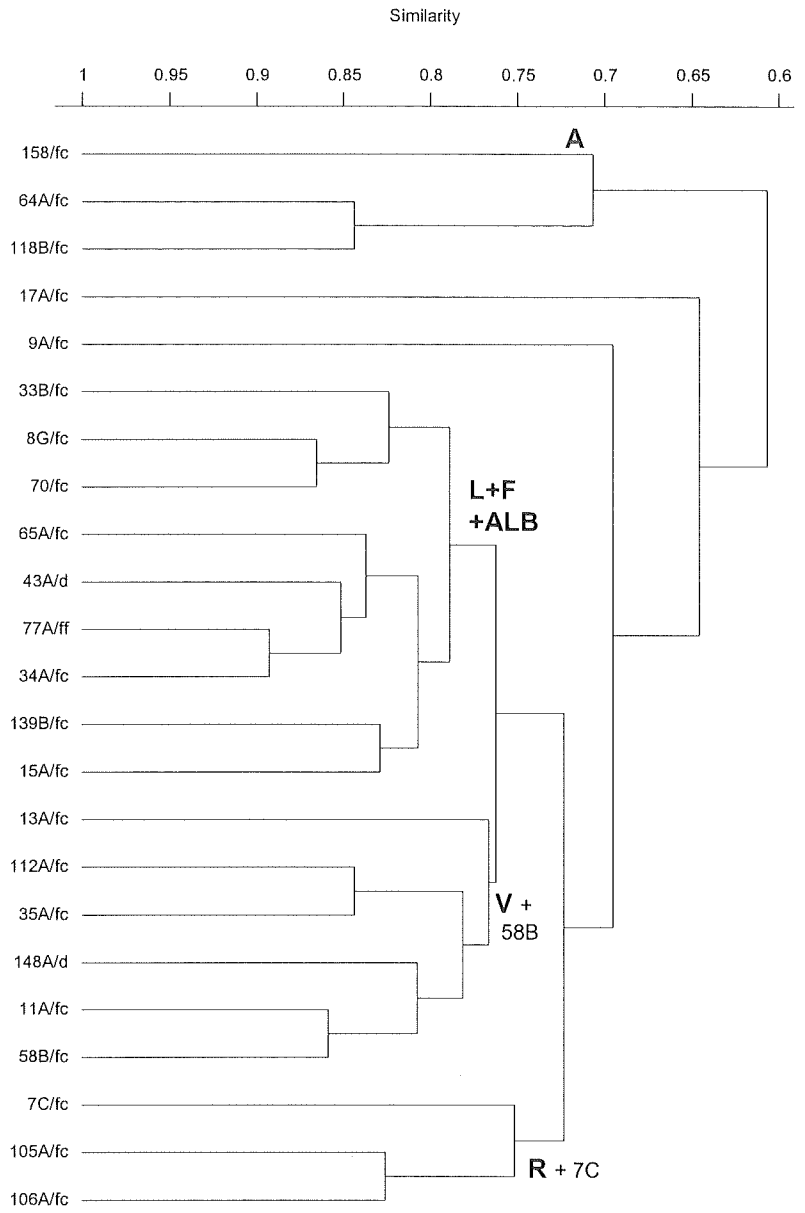


Fig. 2.37 UPGMA phenogram based on Data Set 7.

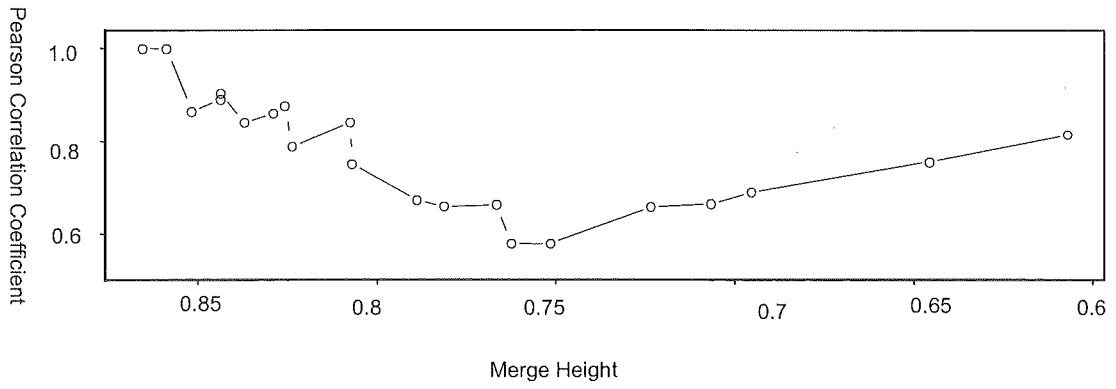


Fig. 2.38 Plot showing the change in the cophenetic correlation coefficient as taxa are clustered in the UPGMA phenogram based on Data Set 7.

2.3.3.4 Numerical analysis of character subsets; vegetative or floral characters

a) UPGMA analysis based on Data Set 8; vegetative characters (characters 1-47)

UPGMA cluster analysis performed on Data Set 8 with 37 vegetative characters resulted in a phenogram (Fig. 2.39), with overall cophenetic coefficient values of 0.7472 (Pearson) and 0.76415 (Spearman Rank). The changes in the cophenetic correlation coefficient as OTUs are clustered in the average linkage phenogram are visualised in a plot (Fig. 2.40).

Six clusters, present at a similarity level of 0.73, were found to resemble the 6 clusters found at the same level based on UPGMA analysis of Data Set 4. Cluster V consisted of all the OTUs grouped in the subcluster Vau of the cluster V in the UPGMA phenogram based on Data Set 4 (Section 2.3.3.2, Fig. 2.22), plus the two additional OTUs 4X/ff and 15A/fc, which moved from cluster Vau, based on analyses of Data Sets 1-3, (Section 2.3.3.1) into cluster F based on analysis of Data Set 4. One representative of 'Retorta' (OTU 108A/fc) was also present in cluster V of the phenogram based on vegetative characters. Cluster V was linked with cluster F at 0.720.

Cluster F comprised all of the representatives of the 'Fulvida' group in a similar arrangement as seen in phenograms based on analysis of Data Set 4. The coastal representatives (7C/fc, 8G/fc, and 5A/d) linked at 0.842 and joined with OTU 77A/ff at 0.818, OTU 34A/fc at 0.794, and OTU 43A/d at 0.746.

Cluster L was found to be identical to cluster L of the phenogram based on Data Set 4, but did not link directly to cluster F, as seen in previous phenograms, but instead joined with cluster R at 0.715. Cluster R contained, besides the remaining representatives of the 'Retorta' group (OTU 105A/d, OTU 105A/fc, and OTU 106A/fc), the herbarium material of the 'Amoena' group and one representative of the 'Albida' group (OTU 159A/d).

Clusters L and R linked at 0.663 with a cluster, in which the second representative of the 'Amoena' group (OTU 158/fc) first became distinct (at 0.710) from the remainder cluster, which then divided into two clusters: ALB containing two representatives of the 'Albida' group (OTU 66B/d, OTU33B/fc), and cluster VP, which is identically to the V subcluster VP of phenogram based on Data Set 4. A similar arrangement of these two clusters could be seen in the UPGMA phenogram based on Data Set 3 (Fig. 2.18).
All clusters were joined at 0.620.

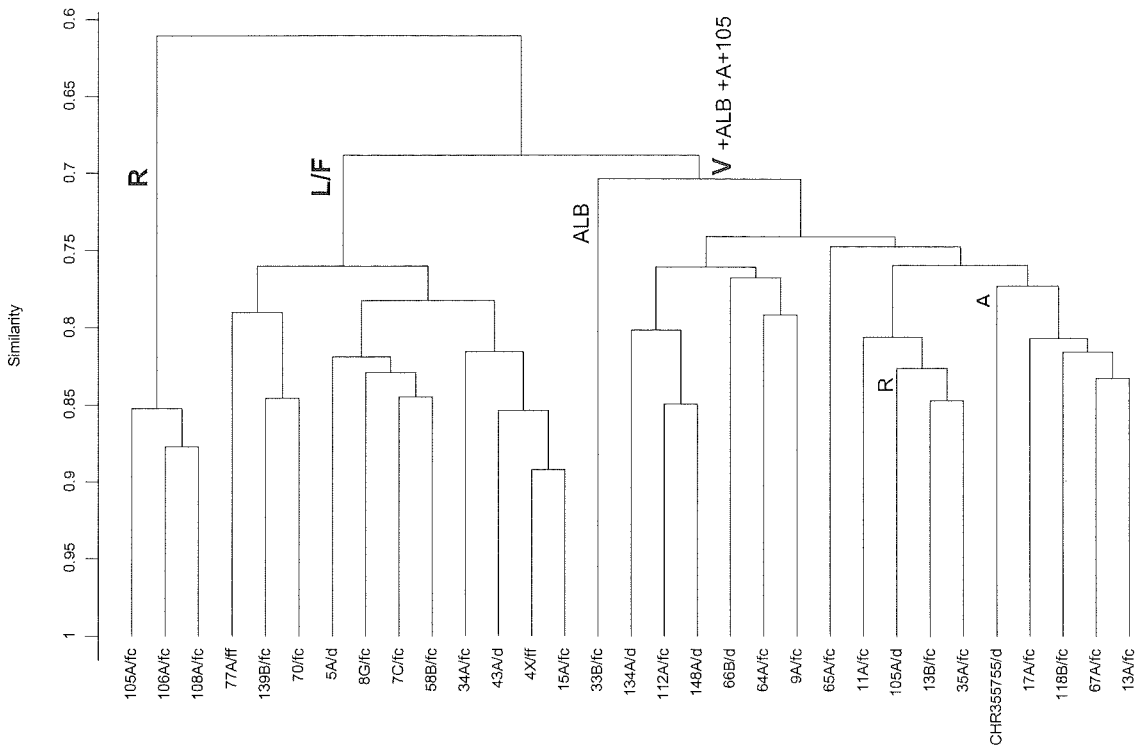


Fig. 2.41 UPGMA phenogram based on Data Set 9 (floral characters only).

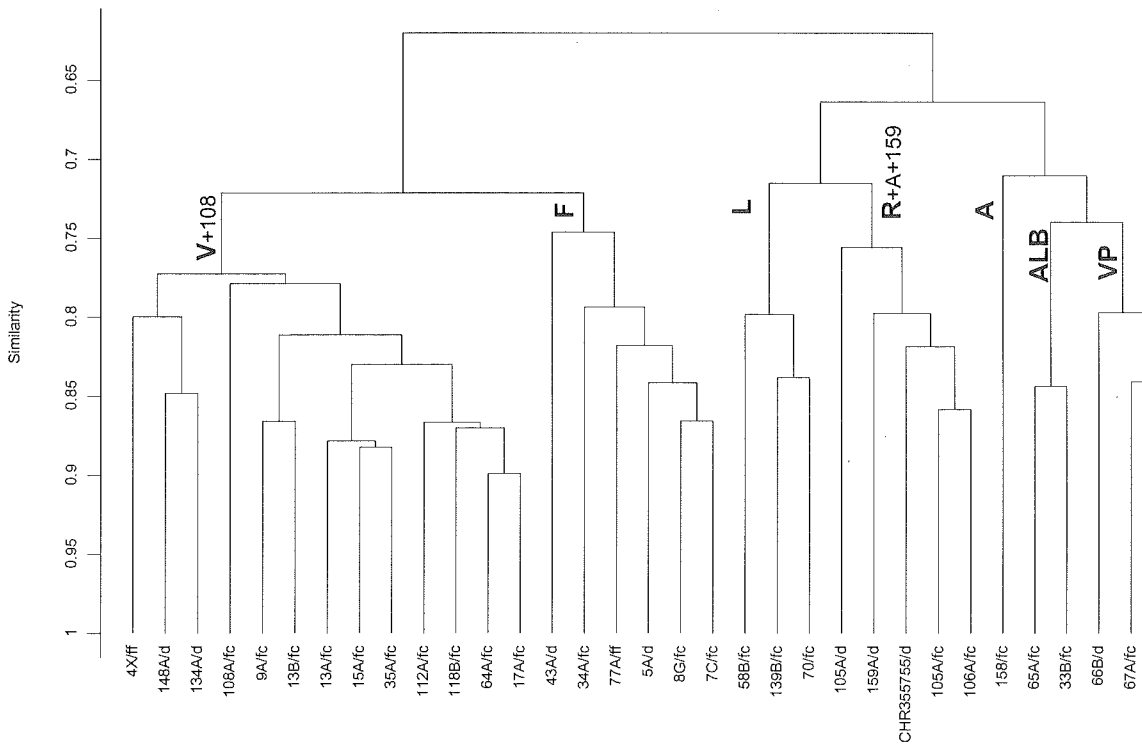


Fig. 2.39 UPGMA phenogram based on Data Set 8 (vegetative characters only).

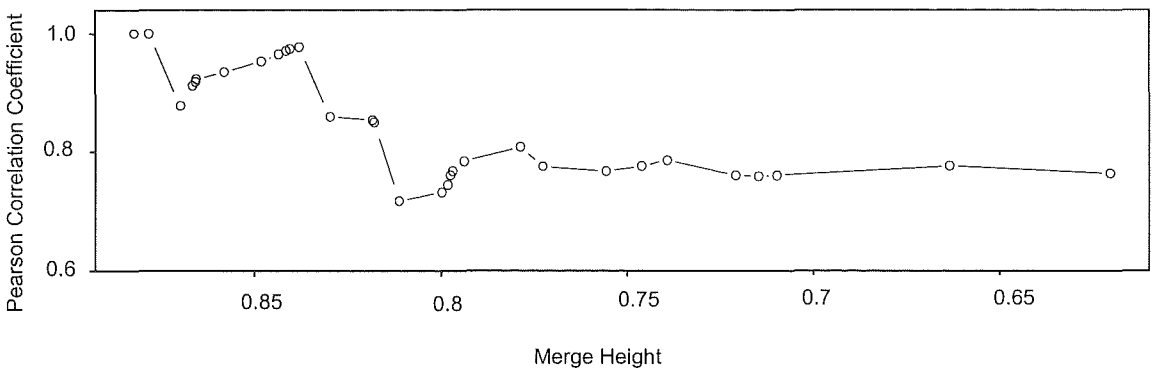


Fig. 2.40 Plot showing the change in the cophenetic correlation coefficient (Pearson) as taxa are clustered in the UPGMA phenogram based on Data Set 8.

Three significant decreases in the cophenetic correlation coefficient could be seen in Fig. 2.40. These decreases occurred when OTUs or subclusters joined within cluster V, e.g., the decrease from 1.0 to 0.875 with the addition of OTU 13A/f to the pair 15A/fc and 35A/fc at 0.878. After increasing again at points of linkage between 0.870 and 0.838, the cophenetic correlation coefficient decreased a second time when the above mentioned cluster joined another at 0.830, containing the OTUs 17A/fc, 64A/fc, 118B/fc, and 112A/fc. The cophenetic correlation coefficient decreased even further to a low of 0.773 when the pair 9A/fc and 13B/fc linked to these clusters at 0.811. It then stabilised with correlations between 0.750 and 0.805 for the links between 0.800 and 0.620.

b) UPGMA analysis based on Data Set 9; floral characters (characters 48-90)

The UPGMA phenogram based on 42 floral characters (Data Set 9) (Fig. 2.41) had overall cophenetic coefficient values of 0.74698 (Pearson) and 0.75159 (Spearman Rank). The change in the cophenetic correlation coefficient as OTUs clustered is shown in Fig. 2.42.

The overall structure of the phenogram appeared to be quite different to the one based on Data Set 4 (Fig. 2.22). Three main clusters could be distinguished. Cluster R was the last to join at 0.611. Cluster L/F comprised all representatives of cluster L/F resultant from analysis of Data Set 4, but these OTUs were shown in a different arrangement. The representatives of the 'Fulvida' group and the 'Leptophylla' group were not separated into different clusters as seen in previous phenograms. Only the representatives of the 'Fulvida Central Otago/Inland Canterbury' group (43A/d, 34A/fc) and the two specimens of the 'Vauvilliersii Cant./Otago/N-Southland' group (4X/ff and 15A/fc) were grouped together

in the same way as in the phenogram based on Data Set 4 (Fig. 2.22).

Cluster L/F containing only small-leaved specimens, linked (at 0.688) to a cluster, containing all larger leaved specimens. OTU 33B/fc was an outlier of this third cluster and joined it at 0.704. Some subclusters showed congruence with those resulting from previous analyses: 13B/fc and 35A/fc linked together strongly (at 0.847 and 0.848 respectively) based on analyses of Data sets 9 and 4. Another cluster present in phenograms resulting from analyses of the Data Sets 9 and 4 included the representatives of the 'Vauvilliersii North Island' group; OTU 112A/fc, linked with OTU 148A/d at 0.85. OTU 134A/d joined this pair at 0.802 in the UPGMA phenogram based on analysis of Data Set 9 and at 0.804 in the phenogram based on Data Set 4. Based on analysis of the floral characters only, the representatives of the 'Albida', 'Amoena' and 'Vauvilliersii var. pallida' groups were scattered within smaller subclusters, without forming distinct clusters. As an example of atypical clustering, one representative of the 'Retorta' group (105A/d) could be found in the cluster linked to the OTU pair 13B/fc-35A/fc at 0.826.

The first significant decrease in the cophenetic correlation coefficient of the UPGMA phenogram based on Data Set 9 (Fig. 2.42) occurred at a similarity level of 0.854, when OTU 43A/d joined the pair 4X/ff and 15A/fc. The cophenetic correlation coefficient stabilised with correlations between 0.88 and 0.94 at the point where links occurred between 0.852 and 0.792. The cophenetic correlation coefficient decreased to 0.81 with the addition of 77A/ff to the cluster formed by 139B/fc and 70/fc and stabilised again with correlations between 0.780 and 0.820 before the last significant drop of 0.08 at 0.741 where the subclusters of cluster V were merged. For the remaining links between 0.704 and 0.611 the cophenetic correlation coefficient remained between 0.69 and 0.74.

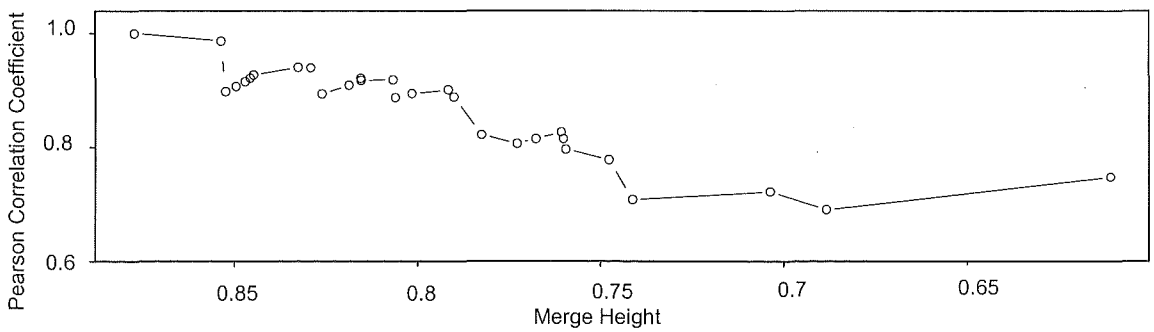


Fig. 2.42 Plot showing the change in the cophenetic correlation coefficient (Pearson) as taxa are clustered in the UPGMA phenogram based on Data Set 9.

Correlations between matrices

The correlation coefficient calculated between the elements of the floral and vegetative similarity matrices using the Mantel test was $r = 0.236$. The correlation of the similarity coefficients was significantly different from zero at the 0.1 per cent level ($p < 0.001$).

2.4 Discussion

2.4.1 Characters

The simplest method of classification involves the formation of groups based on the human capacity for pattern recognition, using the overall appearance of the plants to construct a classification marking character discontinuities between the groups. This is, of course, an extremely subjective method of assessing character discontinuities, and the hopefully more objective and repeatable numerical phenetic approach was used here. The selection of characters for use in a numerical taxonomic study is of greatest importance, since these characters form the basis of the classification. The characters used in the present study have been selected after assessing the variation within the *Ozothamnus leptophyllus* species complex. An attempt was made not to be biased in favour of characters used in previous classifications. Forty-one quantitative characters were assessed by either counting or measurements. States were expressed as the direct measurements and counts. Of the remaining 49 qualitative characters (including one presence/absence character), 46 were ordered qualitative multi-state characters. According to Thiele (1993) and other authors (e.g., Stuessy 1990), there is no difference in nature between quantitative and qualitative characters, as most qualitative characters can be expressed quantitatively, and conversely, any quantitative value can be stated in a qualitative way. Colour characters can often not be arranged in order of magnitude along a one-dimensional axis and have to be treated as unordered qualitative characters. This was, for example, the case for the colour of the outer involucral bracts. Other colour characters used in this study, such as leaf and exudate colour, once divided into the two characters "depth" and "gradient from green to yellow" could be expressed by numerical values that could be ordered.

Logically correlated characters were avoided in character selection, since they

present the same information more than once, a form of unequal weighting. After the discovery that leaf colour and branchlet colour, as well as colour of young and old leaves, were logically correlated, the colour characters for branchlets and old leaves were excluded from the analyses. Correlated characters are clearly not to be avoided as a general principle, since classification is usually based largely on character correlations. The pattern of variation in a character being examined is often correlated with the pattern of variation of other characters. Empirically, highly correlated characters should be included unless they are known to be caused by a single factor.

The fact that most of the characters could be expressed by continuous character states might be seen as an indication of the continuous variation within the *Ozothamnus leptophyllus* species complex. Characters previously used to delimit different *Cassinia* species (Allan 1961) such as leaf measurements, floral counts or number of receptacle scales, varied within populations as well as upon single individuals, but varied less within putative groups than between them.

Not all of the characters are necessarily good identification or key characters, since they may have overlapping values between two or more groups. Identification relies on primary identification characters, which describe a reasonably visible structure, representing a basic property that is not ambiguous in the taxon considered. They also require low variability in the taxon, or at least its variability should be well described and clearly delimited, and there should be ideally a "gap" between the range of character states/values in the specimens that belong to the taxon and the specimens that belong to other taxa. The latter requirement can not always be met since taxa below species level tend to have at least slightly overlapping ranges for most of the character states.

The comparison by a similarity coefficient does not necessarily require a "gap" between the range of character states (Sneath & Sokal 1973). The coefficient is computed on many characters taken simultaneously and OTUs or groups will be statistically distinguished using a combination of characters. This makes numerical methods especially useful on lower taxonomic levels where characters seem to be less distinct between different taxa. The characters used for classification do not have to be easy to observe or measure (Sneath & Sokal 1973).

The value of the characters will be discussed following the discussion of the results of the numerical analysis.

2.4.2 Cluster analyses and ordination

Use of cluster analysis in numerical taxonomy is most often applied in situations in which the groups are unknown prior to analysis. Hierarchical methods in which clusters are defined according to similarity or dissimilarity measures (Section 2.2.3.2) are the most common methods of analysis, and were used in this study to detect groups within the sample group, and to estimate overall similarity among these groups.

There are many clustering methods, of which single linkage, complete linkage, the unweighted pair group method using arithmetic averages (UPGMA), and the weighted pair group method using arithmetic averages (WPGMA) were applied and evaluated in this study. These methods influence ways in which clusters are formed, and when different clustering techniques are applied to the same similarity or distance matrix the resulting phenograms often differ slightly in the content of the clusters and the levels at which the clusters form. This can be explained by the nature of the data and how the different algorithms calculate the clusters (Section 2.2.3.2). Differences tend to occur when there are no natural clusters in the data, or as in the present study, distortions occur due to the presence of hybrids or intermediate forms (as was also found by Jardine 1969). Variability in cluster composition and position was most evident in this study during analyses of 1) the complete data matrix containing 192 OTUs (Data Set 1), and 2) the slightly reduced matrix which still contained most of the representatives of the polymorphic populations (putative hybrids) (Section 2.4.4). Phenograms formed by single linkage clustering for these two matrices did not show a hierarchical structure. A marked chaining effect is apparent with individuals or small clusters tending to join one by one to a larger established cluster rather than beginning to form their own cluster. Some homogeneous populations were represented by several OTUs, which clustered together at high similarity levels leading to uneven initial cluster formations. The presence of intermediate forms between plants of the 'Vauvilliersii', 'Leptophylla', and 'Fulvida' groups were most likely responsible for preventing the formation of clear distinct clusters in these earlier analyses. The single linkage phenograms based on Data Set 1 showed the worst fit to the similarity matrices indicated by the lowest overall cophenetic correlation values found in this study. The overall cophenetic correlation value for the single linkage phenogram based on Data Set 2 was also very low but very similar to the values for the complete linkage phenogram based

on that data set.

The cophenetic correlation coefficient of Sokal and Rohlf (1962) is a measure of the degree of fit of each phenogram to the similarity matrix. It can also be used as a measure of degree of fit of a phenogram to a set of data and as a criterion for evaluating the effectiveness of various clustering techniques. It indicates the extent to which different clustering methods distort the original data or a set of pairwise similarities (or dissimilarities) derived from them. The cophenetic correlation coefficient can therefore be seen as a measure of information content for a phenogram. The objectives of numerical taxonomy are largely concerned with constructing taxa based on maximum information content, which require phenograms with high cophenetic correlation values. Farris (1969) warned about employing the cophenetic correlation coefficient as an optimality criterion because it is not a direct measure of the degree to which a phenogram describes the distribution of character states. According to Farris (1969) the choice of an optimality criterion for classifications has to be determined in conjunction with the choice of a measure of similarity between OTUs and an implied measure of information content of classifications. McNeill (1979) agreed that measures such as the cophenetic correlation coefficient have a role in assessing the merit of one phenogram relative to another but argued in support of Farris (1969), that clustering is not only a matter of best ultrametric transformation, but also the production of an acceptable simplification that reflects to the best possible degree the original relationships of the taxa. Of importance according to McNeill (1979) is the concept of structural value. This means that a symmetrical hierarchical structure is a very desirable characteristic of a phenogram.

For the analyses of Data Sets 1 and 2 based on complete linkage clustering, a clear hierarchical structure was evident in each of the phenograms. But through comparison of results obtained from different clustering methods, it became clear that the complete linkage phenogram based on Data Set 1 resulted in outlying OTUs being forced into associations with distant partners. The contents of the clusters in the complete linkage phenogram based on Data Set 2 resembled the contents of the clusters in the UPGMA and WPGMA phenograms but structural differences were apparent. The cluster formed by representatives of the 'Amoena' and 'Retorta' groups, present in an outlier position in the single linkage phenogram as well as in the UPGMA and WPGMA phenograms, was linked with the 'Vauvilliersii' cluster, before joining the 'Leptophylla/Fulvida' cluster. Low

cophenetic correlation values for the complete linkage phenogram based on Data Set 2 indicated a high distortion in the cluster arrangement.

UPGMA and WPGMA clustering for Data Set 1 resulted in very similar cluster arrangement, producing well structured phenograms with the highest overall cophenetic correlation values of all phenograms produced based on Data Set 1 (0.75 and 0.72 respectively). This supports the observation of Sokal and Rohlf (1962) that phenograms formed by average clustering methods show the best fit.

Representatives of the 'Amoena', 'Albida' and 'Retorta' groups were, in both UPGMA and WPGMA phenograms, distantly linked together. They formed a main cluster linked to two additional clusters, one comprising representatives of the 'Leptophylla/Fulvida' groups (small leaved group) and the other representatives of the 'Vauvilliersii' group (big leaved group). However, distortion became obvious if subclusters were examined and compared more closely. Some representatives of the 'Vauvilliersii var. pallida' group showed affinities to intermediate OTUs from polymorphic populations and formed a subcluster within the V cluster of the UPGMA phenogram. This entire subcluster could be found within the L/F cluster of the WPGMA phenogram where it linked with other representatives of polymorphic populations.

By comparing the UPGMA and the WPGMA phenograms based on Data Set 2 the distortion due to representatives of the polymorphic populations became even more obvious. Clusters containing representatives of polymorphic populations could be found linked with different clusters in the UPGMA and WPGMA phenograms, causing, for example the appearance of the cluster VP ('Vauvilliersii var. pallida' group) in different main clusters. Cluster VP was linked to the representatives of the cluster ALB within cluster A+ALB in the WPGMA phenogram, while it was a subcluster of the 'Vauvilliersii' cluster in the UPGMA phenogram (Section 2.2.3.1). Differences between these two phenograms were also expressed in different cophenetic correlation coefficients. While the overall cophenetic correlation coefficient for the UPGMA phenogram was 0.71, the value for the WPGMA phenogram was only 0.61. The eight phenograms produced from analyses of the complete data matrix containing 192 OTUs (Data Set 1) and the slightly reduced matrix containing 112 OTUs (Data Set 2) gave a very good first impression about the nature of the data, and visualised the occurrence of intermediate forms and putative hybrids. However, it is the very presence of these intermediate forms that makes it difficult

to identify distinct groups within the *Ozothamnus leptophyllus* complex (Webb 1988). Due to the large number of OTUs included in Data Sets 1 and 2, and the distortion encountered, the analyses of these two data sets were used primarily to reduce the number of OTUs. The actual determination of taxa was based on the analysis of the reduced Data Sets 3 and 4 using again the four different clustering methods.

When the four different clustering techniques were applied to the same similarity matrix generated from Data Set 3, the resulting phenograms showed minor differences in the content of the clusters and the levels at which the clusters formed. The values of cophenetic correlation coefficient indicated that the cluster methods produced decreasing cophenetic correlation coefficients, from the best fit generated by UPGMA clustering, followed by WPGMA clustering, single linkage clustering, and finally complete linkage clustering, which showed a relatively poor fit.

The distortion that became obvious by comparing the different clustering results was important for describing the phenetic relationships and the "distinctiveness" of clusters. This also aided interpretation of results of analyses of the even more reduced Data Set 4. The OTUs 65A/fc, 159A/d and 33B/fc, forming one of the main clusters (ALB) in the UPGMA, WPGMA, and complete linkage phenograms based on Data Set 4, showed affinities to the representatives of the 'Vauvilliersii' subcluster VP in the UPGMA phenogram based on Data Set 3. These two subclusters linked with each other in the WPGMA and the complete linkage phenograms based on Data Set 3, but no longer as a subcluster of the V cluster; i.e., they joined with the 'Amoena' and the 'Retorta' cluster. The single linkage phenogram based on Data Set 3 identified OTU 65A/fc and 33B/fc clearly as outliers. However the strong affinity of these OTUs to some representatives of the 'Vauvilliersii var. pallida' (VP) subcluster as seen in the other three phenograms based on Data Set 3 should not be overlooked, and indicates the presence of intermediate forms between these two groups.

The differences in the cophenetic correlation values of the phenograms based on Data Set 4 were smaller. Although the UPGMA had again the highest value, the complete linkage and the single linkage phenograms showed nearly equally good fit. The WPGMA phenogram had the lowest cophenetic correlation coefficient. The cluster contents of the WPGMA phenogram were identical to the phenograms formed by UPGMA and complete linkage clustering of Data Set 4, and the cluster arrangement differed only slightly. The

cophenetic values for the different linkages of clusters within the different phenograms indicated that the UPGMA phenogram represented the best fit. The phenograms differed also in the levels at which the clusters formed. For the designation of the "main clusters" or phenons (Sneath & Sokal 1973), the UPGMA phenogram was chosen because of its high overall cophenetic correlation value. A phenon line was drawn at similarity level of 0.735, where the cophenetic correlation coefficient stabilised around 0.75. In the WPGMA phenogram as well as in the complete linkage phenogram, a line could also be drawn, deliminating the same clusters as in the UPGMA phenogram. In these latter phenograms, this line was sometimes very close to linkage points of clusters and its positioning might be seen as not justifiable as a phenon line, which is meant to be drawn objectively and not to fit preconceived groups (Sokal & Sneath 1963). In the complete linkage phenogram, the clusters formed over a wider range of similarity values compared to the UPGMA and WPGMA phenograms, due to the more stringent clustering requirements. In this case the line to distinguish the same "main cluster" had to be drawn at a similarity level between 0.66 and 0.68 to distinguish between the A and the ALB cluster, but not to split the R cluster. No line was drawn in the single linkage phenogram because the clusters formed within a narrow range of similarity values and a chaining effect was apparent. The single linkage clustering highlighted outliers such as OTU 65A/fc, 66B/d, and 33B/fc, and visualised affinities of representatives of the 'Fulvida' group to the 'Vauvilliersii' group. 'Amoena', 'Retorta', 'Leptophylla' and the coastal representatives of the 'Fulvida' group also formed distinct clusters with single linkage clustering.

Interpretation of cluster analysis results can often be highly subjective. The validity of the conclusions drawn from cluster analysis techniques is sometimes questioned since very different clusters can be formed from the same data depending on how the analysis is performed. Therefore, it is advisable to explore different methods and to compare the results. Clusters can then be determined from one of the clustering methods as was carried out in the present study, based on the cophenetic correlation coefficient, and subsequently distortion can be detected. It is also important to check the clusters manually against the similarity matrix to detect distortion of phenetic relationships. Discrepancies in placement of OTUs when they are compared to their similarity values are usually indicated by low cophenetic correlation values for the particular linkages.

Ordination techniques are not required to produce a tree-like hierarchy, so distortion created by this constraint is not present. Even if ordination techniques usually do not lead to an explicit separation of OTUs into groups and therefore do not produce classifications (Dunn and Everitt 1982), they are very useful for indicating the taxonomic structure in a collection of organisms and for investigating the general pattern of variation (Sneath & Sokal 1973). Sneath and Sokal (1973) suggested ordination techniques as a suitable complement to cluster analysis. Particularly at the infraspecific level ordination techniques are very useful (e.g., Jensen & Eshbaugh 1976a and 1976b, Schilling & Heiser 1976, Thorpe 1983, Section 2.4.4), especially in cases where the data show poor hierarchical structure (Jardine 1969). In Section 2.4.4 the use of principal coordinate analysis (Gower 1966) for detecting putative hybrids and intermediate forms in Data Set 2 will be discussed.

The principal coordinate graphs based on Data Set 4 not only highlighted the grouping of OTUs which were found to form clusters in the UPGMA phenogram, but also illustrated the overlap between clusters and identified intermediate forms and outliers. Based on cluster analysis, the OTUs 15A/fc and 4X/ff, both from the mountain areas in Canterbury and originally assigned to the 'Vauvilliersii Cant./Otago/N-Southland' group, were suspected to have an intermediate status between 'Vauvilliersii' and 'Fulvida'. Principal coordinate 2 showed these two OTUs between the 'Vauvilliersii' and the 'Fulvida' groups. Based on principal coordinate analysis, the three representatives of the 'Vauvilliersii var. pallida' group 66B/d, 67A/fc and 11A/fc formerly treated as *Cassinia vauvilliersii* var. *pallida* (Allan 1961), could be identified as intermediate forms between the 'Vauvilliersii' groups and representatives of the 'Albida' group. These latter OTUs, formerly described as *Cassinia vauvilliersii* var. *albida* (Kirk 1899) or *Cassinia albida* (Cockayne 1906), were together with 66B/d, 67A/fc and 11A/fc originally assigned to the 'Vauvilliersii var. pallida/albida/canescens' group. Principal coordinate 3, explaining 8.6% of the variation in the data set, clearly identified the 'Retorta' and the 'Albida' groups as distinct, the latter with some affinity to the 'Amoena' group as outliers.

2.4.3 Reduction of the OTU number

The objective of this section of the study was to reduce the initial number of 192 OTUs used in numerical phenetic analysis down to 23-25, reflecting the number of OTUs used for other areas of investigation, i.e., micromorphology and molecular data; thereby allowing direct comparison among data sets.

Phenograms are more accurate near the tips, where relationships among highly similar OTUs are portrayed (Crovello1968c, Sneath & Sokal 1973). Closely-linked OTUs are very similar to each other and their linkage is strongly supported by high cophenetic correlation values, so that one OTU of each of the most accurate first level clusters can be used to represent the whole cluster. In a sequential reduction procedure, involving three steps (2.2.3.5), OTUs which clustered on a high level of similarity with other OTUs, were excluded from further analysis. Thus was based on the fact that the clustering procedure leading to the phenogram began with OTUs that possess the maximum mutual similarity and groupings at the initial level of clustering are less influenced by distortion.

When analyses of Data Sets 1-4 (Section 2.3.3.1 and 2.3.3.2) were studied in sequence, certain generalisations could be made. Among the UPGMA phenograms based on Data Set 1, 3 and 4, the overall cophenetic values remained very similar (~ 0.75). The UPGMA phenogram based on Data Set 2 had a slightly lower cophenetic values (0.71). The presence of representatives of polymorphic populations caused distortion in the phenograms based on Data Sets 1 and 2 (Section 2.4.4) which was indicated by a lower overall cophenetic correlation value for the UPGMA phenogram from Data Set 2. The overall cophenetic correlation coefficient for the phenograms based on Data Set 1 seemed to be less affected by the presence of representatives of polymorphic populations. This phenogram included a greater number of very accurate links, supported by high cophenetic values, due to the presence of more OTUs that were linked on a high level of similarity. After removing such links by excluding 80 OTUs from the analysis of Data Set 2, the overall cophenetic correlation coefficient dropped.

The cophenetic correlation coefficient depends not only on the method producing the phenogram but also on the natural structure of the OTUs being classified (Sneath & Sokal 1973). It can be seen as a function of the pattern of OTU variation among each of the four data sets. The consistency of the overall cophenetic correlation coefficient for the

phenograms might be interpreted as an indication that with further reduction of the number of OTUs the depicted variation would remain the same.

Four main clusters (R, A, V, and L/F) appeared in all analyses and could be regarded as constant in terms of cluster structure. The number of smaller clusters decreased with the reduction of the number of OTUs, but the content and relationships within larger clusters remained unchanged, with two exceptions: OTU 4X/ff and 15A/fc were placed within the VCOS cluster, one of the 3 subclusters of the cluster Vau of the UPGMA phenogram based on Data Set 1. After exclusion of OTUs from this subcluster (Data Set 2), the OTUs 4X/ff and 15A/fc still remained within the VCOS cluster and formed a subcluster with OTUs 124F/fc, 15B/fc, and 163A/ff. This procedure was repeated with the removal of OTUs 124F/fc, 15B/fc, and 163A/ff (Data Set 3), the OTUs 4X/ff and 15A/fc then appeared in the 'Fulvida' cluster forming a subcluster with the representatives of the 'Fulvida Central Otago/Inland Canterbury' group (OTU 43A/d, and OTU 34A/fc). The reduction of the number of OTUs thus caused 4X/ff and 15A/fc to be placed in a different main cluster.

The OTUs 65A/fc, 159A/d and 33B/fc formed a single cluster, except in the UPGMA phenogram based on Data Set 2 where OTU 159A/d was linked to representatives of a polymorphic population (3B/d, 3A/f). These OTUs, assigned to the 'Albida' group, were linked distantly to the representatives of the 'Amoena' group in the UPGMA phenograms based on Data Sets 1, 2, and 4. In the UPGMA phenogram based on Data Set 3 they could be found within the 'Vauvilliersii' cluster, linked to representatives of the 'Vauvilliersii var. pallida' group.

The rearrangement of OTUs in different main clusters due to reduction of the number of OTUs might be explained as follows. With each new analysis, OTUs were removed which may have been weakly linking 'intermediate' OTUs together. A comparison of similarity matrices (Appendix 8) showed that the OTUs 4X/ff and 15A/fc were both more similar to the OTUs 34A/fc and 43A/d than to the OTUs grouped together within any of the 'Vauvilliersii' subclusters, excluding some representatives of the VCOS cluster, which were removed from further analysis. The appearance of the representatives of the 'Albida' group in the 'Vauvilliersii' cluster in the phenogram based on Data Set 3 can be explained in a similar way. The removing of the representatives of the polymorphic populations from the analysis, which were linked with the representatives of the 'Albida'

group in the phenogram based on Data Sets1-2, led to the arrangement of OTUs 65A/fc, 159A/d and 33B/fc within the 'Vauvilliersii' cluster forming a subcluster linked to the VP cluster. A high similarity value between OTU 159A/d and OTU 11A/d caused this rearrangement. After excluding OTU 11A/d, the 'Albida' group again joined the 'Amoena' cluster on a low level of similarity (UPGMA phenogram based on Data Set 4).

Little could be found in the literature on the effect of change of OTU numbers on the resulting clusters. However, Crovello (1968c) made very similar observations to the present study by adding OTUs to a data set and comparing the resulting cluster analyses. Using 28 taxospecies of the genus *Salix* in California and adding different numbers and subsets of 22 OTUs of the section *Sitchenses*, which was already represented within the 28 taxospecies, he found that the number of OTUs used in a study does make a difference in cluster analysis. The encountered difference was not random but centred on those OTUs that are most closely related to the OTUs being added.

Sneath and Sokal (1973) suggested that the addition or removal of OTUs does not have serious consequences in determining taxonomic rank from analyses. They remarked that the methods of cluster analysis employed would themselves have some influence on the ranks, since different methods summarised the similarity matrix in slightly different ways, and that this effect would be as great as that produced by omitting a small proportion of OTUs.

2.4.4 The inclusion of representatives of polymorphic populations (putative hybrids)

Clustering can provide clues to the identification of putative hybrids and their parents, but ordination techniques have been shown to be better suited for the display of intermediate groups (e.g., Jensen & Eshbaugh 1976a and 1976b, Schilling & Heiser 1976). Evidence for distortion of clusters due to the presence of suspected hybrids often comes from their intermediate positions between specimens of different distinct groups (Schilling & Heiser 1976).

Individuals tentatively identified as hybrids (representatives of polymorphic populations) in this study tended to form distinct sub-clusters, which were arranged within

one or the other main cluster containing representatives of different populations (putative parents). Similar results were found by Jensen and Eshbaugh (1976b) in their numerical taxonomic study of hybridisation in *Quercus* populations, where putative hybrids produced either a distinct cluster or joined another cluster at a relatively low level of similarity. However, their samples represented populations with a wide distribution and high taxonomic diversity. Heiser et al. (1965) and Bemis et al. (1970) showed that hybrids of widely divergent taxa typically display higher correlations with nonparental taxa than with parental taxa. An estimation of the origin of putative hybrids can be difficult in populations with many potential parental taxa, especially those containing both very similar and widely divergent taxa (Jensen & Eshbaugh 1976b). Nevertheless, numerical taxonomy can be useful in predicting the probable parentage of naturally occurring putative hybrids if they are from populations with low taxonomic diversity and narrow geographic distribution, as shown by Jensen and Eshbaugh (1976a). The results of their study of three populations of red oaks (*Quercus* subgenus *Erythrobalanus*) that met the above criteria supported the hypothesis that hybridisation can be detected by the intermediate characteristics of putative hybrids.

Jensen and Eshbaugh (1976a) found that representatives of hybrid swarms not only assumed intermediate positions, but some were extremely close to one parent. Whiffin (1973) suggested introgressive hybridisation as the controlling factor for similar behaviour that he encountered in some of his putative hybrids between *Heterocentron elegans* and *H. glandulosum*. This could also apply to representatives from polymorphic *Ozothamnus* populations examined in this study. The wide range of variation encountered in polymorphic *Ozothamnus* populations might be also due to F_2 segregation. Breeding within a F_1 generation could have led to a very variable F_2 generation in which the range of variation includes the appearance of one or both parents or displays new combinations of characters.

Principal coordinate analysis showed that the polymorphic population from Ward beach in Marlborough (Poly1) contained specimens which were positioned intermediate between the 'Fulvida' and the 'Leptophylla' groups, but on occasion clearly were closer to one or the other of these groups. Less clear was the assessment of populations such as Poly2 ranging from the Chalk Range to the Remuera Station, below the Chalk Range in Marlborough. Besides intermediate individuals, this population contained some specimens

that were closer to representatives of the 'Fulvida' group, some closer to the 'Leptophylla' group, and others which clustered within the 'Vauvilliersii var. pallida' group. This same situation was found for the polymorphic population from the Isis Stream valley, Marlborough (Poly4), which also contained intermediate individuals, as well as some which grouped closer to or within the 'Fulvida', 'Leptophylla', or the 'Vauvilliersii var. pallida' groups. Putative hybrids from populations such as those described from Marlborough, where three different groups overlap geographically, may be the result of crossing among three distinct entities. The polymorphic populations Poly3 (Parara Wetland, Marlborough) and Poly5 (Hodder River valley, Marlborough) have not been sufficiently sampled. However, the representatives of these populations were either positioned intermediate (Poly3) or with some affinities to the 'Fulvida' group (Poly5) based on principal coordinate analysis.

Further studies are needed to assess hybridisation and gene flow between different *Ozothamnus* populations, before plants may be positively identified as being of hybrid origin. Intermediate forms have been recorded in many instances from regions were 'distinct forms' are overlapping. In the literature, such forms have been described as hybrids. For example Kalin (1967b) studied the problem of continuous variation and hybridisation in the overlapping coastal *Ozothamnus* population previously treated as different species '*Cassinia fulvida*' and '*C. leptophylla*'. In their region of overlap, which extends from Cloudy Bay to the Flaxbourne River, the "species" form a wide band of hybrid populations, occupying coastal habitats. Carse (1930) described *C. leptophylla* x *retorta* and *C. retorta* x *amoena*, and Cockayne and Allan (1934) listed *C. albida* x *fulvida* var. *montana*, *C. albida* x *vauvilliersii*, *C. fulvida* var. *montana* x *vauvilliersii* and *C. retorta* x *vauvilliersii*.

2.4.5 The inclusion of OTUs for which a large number of characters are missing

Vegetative samples were occasionally collected in the field from individuals lacking flowers, or having old or immature flowers. When a character state was missing it was labelled with NA for "not applicable". Such characters are omitted in the calculation of the similarity coefficient (Gower 1971, Sneath & Sokal 1973). This has the effect that

confidence limits of the similarity values within a similarity matrix will vary depending on the amount of missing data. The statistically significant difference between two coefficients in a similarity matrix may be nonsignificant between another two coefficients in the same matrix, based upon a smaller sample of characters and the coefficients used (Sneath & Sokal 1973).

The fact that incomplete field specimens and complete cultivated specimens from the same plant did not always link directly with each other in the present study might have been caused by missing data. For example, the two representatives of a homogeneous population, OTU 65A/d and OTU 65B/d, both field specimens without floral characters, were linked on a very high level of similarity while the cultivated material of OTU 65A with a complete set of characters was linked with a third representative of the population, OTU 65C/d, which was collected in the field but with floral data (refer to Fig. 2.13). Other OTUs which caused a slight distortion in the phenograms based on Data Set 1, due to a large number of missing values included, 158/fc, 158/ff, 159A/d, 11B/d, 33B/d, 7C/d, 52A/d, 58B/d, 70/d, 112A/d, 156A/ff, 76K/d, 76J/d, 76A/d, 15A/d, 35A/d, 36A/d, 105D/d, 106A/d, and 106B/d.

Most OTUs with missing data clustered on a high level of similarity with either cultivated material of the same plant (if available), or with representatives of the same populations or groups. Such OTUs were then excluded from further analyses. Only 2 OTUs, OTU 105A/d and OTU 159A/d, with no floral characters were carried through all the steps of reduction (Section 2.2.3.5) and remained in the reduced data set (Data Set 4). OTU 105A/d, a representative of the 'Retorta' group, clustered within the R cluster of the UPGMA phenogram based on the reduced Data Set 4, but was not directly linked with the complete cultivated material of this plant (OTU 105A/fc). Only incomplete old flowering material was available for OTU 105A/d, which might also explain the placement of this OTU in the phenogram based on floral characters only (Data Set 9). OTU 159A/d, a representative of the 'Albida' group, was maintained in the analysis despite the missing floral characters because it was also included in the molecular studies. This OTU clustered with the other representatives of the 'Albida' group in the UPGMA phenogram based on Data Set 4, but could be found within the R cluster in the phenogram based on Data Set 5. Data Set 5 contained 15 additional microcharacters. The microcharacters were from floral parts only, and these were not available for OTU 159A/d. Therefore the relevance of

comparison of OTU 159A/d with other OTUs with a complete set of data was even lower within Data Set 5 than it was in previously data sets without the microcharacters. The relevance of a comparison can be expressed with a relevance value:

$$R_{jk} = a_{jk}/n$$

Where a_{jk} is the number of characters applicable in OTUj that are also applicable in OTUk, and n is the number of characters employed in the study (Sneath & Sokal 1973). The OTU relevance for comparing OTU 159A/d with any OTU with a complete character set in Data Set 5 was 0.406 (compared with 0.463 in Data Set 4).

OTU relevance can provide a guide to the reliability of the position of an OTU in a phenogram. Thus if an OTU has a relatively low relevance value, then its position in a phenogram should be considered to be less reliable than the position of OTUs with higher OTU relevance (Crovello 1968a). High correlation coefficients between similarity matrices based on characters with complete or almost complete entries (high mean OTU relevance) and matrices based on characters with up to half the entries missing (low mean OTU relevance) indicate that numerical analysis seems to be fairly robust, and can tolerate a good amount of low relevance and missing data (Crovello 1968b). However, as shown in this study and in others of its kind (e.g., Ward 1981), missing data can distort relationships, and the number of "not applicable" entries should be kept at a minimum.

2.4.6 Comparison of herbarium (dried), fresh field, and fresh cultivated material using numerical techniques

All characters selected for this study were retained in dried herbarium material and could be measured and assessed if the herbarium material was prepared from specimens with both floral and vegetative parts. Measurements such as branchlet and leaf angles resulted in very similar mean values (Section 2.3.2), within the standard error when taken from both herbarium and fresh material from the same plant. However, depending on the size of the herbarium material, it was not always possible to get ten branchlet angle measurements as only branchlets that were still in natural positions could be measured.

The characters that changed the most when specimens were dried were the colour characters of the leaves, an observation also made by Kalin (1967b). Exudate colours were

found to be unaffected by drying, but the leaves of dried specimens were usually slightly darker, with a higher value for the gradient green to yellow compared to fresh material. It is likely that the colour differences between dried and fresh material, as well as missing values (as discussed above), contributed to the fact that dried and fresh material of the same plant did not always link directly with each other. Some dried specimens clustered on a high level of similarity with other herbarium material from the same population or group (Section 2.3.3.1), possibly due to the fact that the leaf colour of a dried specimen might be more similar to the leaf colours of another dried specimen of the same homogeneous population, than to the leaf colour of a fresh specimen of the same plant.

In certain circumstances, floral characters could not be assessed directly from dried specimens, especially those that vary according to atmospheric relative humidity, e.g., the diameter of the capitula and the degree of spread for the outer and inner involucral bracts. Thus it became necessary to rehydrate material first. Generally rehydrated material produced similar results to fresh specimens.

A direct comparison between fresh field material and dry field material was possible for only a very small number of specimens, for example OTU 17A, and OTU 8G, which were assessed before they were pressed and dried. Most field collections were turned into herbarium specimens before assessing the characters.

During the course of this study it became necessary to compare herbarium specimens collected in the field with fresh cultivated specimens. The question was therefore not only how much the process of drying modified the characters, but also if certain character states expressed environmentally induced variation. This process is not straightforward and it is not always possible to determine the nature of variation, whether genotypic or environmentally induced, in the herbarium or even in the field. Comparative cultivation is therefore necessary to detect phenotypic plasticity. However, most of the characters selected for examination of variation within the *Ozothamnus leptophyllus* complex remained unchanged in cultivation, and differences between populations and groups persisted in cultivation. This was shown in the numerical analysis based on the Data Sets 1-3 (Section 2.3.3.1), that contained field collected and cultivated material of the same plants, both of which were always present in the same cluster and joined frequently on a high level of similarity.

Only a few characters studied from field collected material were found to be

influenced by environmental factors (refer to Section 2.3.2). The growth habit of plants originally from extreme habitats e.g., unstable sand dune systems or above the tree line on mountain ridges, changed in cultivation from very small decumbent or prostrate to taller more upright. Persistent wind action might have caused this phenotypic modification. Most of the other character states seemed to be genetically fixed as they remained unchanged in cultivation, for example the short-stemmed, fewer-flowered (smaller panicles) mountain forms remained unchanged in cultivation and were clearly distinguished from the taller lowland *Ozothamnus* plants with large panicles.

Obvious phenotypic plasticity could sometimes be detected in the field. Knowing the natural distribution and typical habitats of *Ozothamnus leptophyllus* (Section 1.3), and considering that *O. leptophyllus* has a high light intensity requirement, plants occasionally found in unusual localities such as open bush and forest areas were excluded from the character assessment. These specimens did not flower and showed quite marked variations in form and habit caused by the lack of light, such as stem elongation, loose branching patterns and a long internode length. Biotic factors such as the effect of insect attacks destroying floral material or fruits, or leading to the forming of galls (Section 1.4.1.2) were also easily recognised and were seldom likely to cause difficulties of taxonomic interpretation.

2.4.7 Analysing floral and vegetative characters separately

Sokal and Sneath (1963) proposed a hypothesis of nonspecificity which, if true, would require that classifications based upon different sets of characters would differ no more than could be accounted for by chance. Because there are no large independent classes of genes, any character sample will give the same indication of similarity among a group of organisms as any other sample (Sneath & Sokal 1973). Comparison of similarity matrices and phenograms based on *Ozothamnus leptophyllus* floral and vegetative data sets (combined and separate) does not support this hypothesis. Highly significant, but not large correlations ($r = 0.236$) were found between vegetative and floral data sets when analysed using the Mantel (1967) test.

The smallest published correlation found during a literature search between two data sets was for floral and vegetative characters in the milkweed *Sarcostemma* ($r = 0.169$)

(Johnson & Holm 1968). Rohlf (1963) explained similar results that he encountered as being due to a "lack of complexity" in the relationships among OTUs. This is in discordance with the results of Ward (1981). The correlation between the similarity matrices based on floral characters and vegetative characters of *Raoulia* ($r = 0.58$) was slightly higher than that for the Gnaphaliinae ($r = 0.52$), even though the relationships within the Gnaphaliinae are more complex than those within *Raoulia*.

However the present study as well as several others (e.g., Rohlf 1963, Ehrlich & Ehrlich 1966, Johnson & Holm 1968, Ward 1981) showed that different sets of characters often led to different sets of relationships. A biological interpretation of these results would be that the effects of a large proportion of the genes are limited to a single body region (or life-history stage (Rohlf 1963)). Therefore, characters sampled from any one body region are not direct estimates of the overall relationships, but there are a sufficient number of genes with major effects in both the vegetative and the generative plant parts so that the resulting classifications are not completely independent. Another possible explanation would be that evolutionary divergence or convergence occurs in one of the principal functional areas of the angiosperm plant body, without necessarily occurring in the others. Therefore two groups of plants may, for example, be very similar in leaf characters but very different in floral characters due to similar selection pressures for light absorption, transpiration and photosynthesis, but different ones for pollination and seed dispersal. This suggests the necessity for a re-examination of the question of what kind of character sampling is needed to produce a general classification. Rohlf (1963) recommended that an effort should be made in a numerical taxonomic study to record as many different kinds of characters as is practical, and to try to distribute them evenly between "the two sexes, among the life-history stages, internal versus external anatomy, body regions, etc." (Rohlf 1963: 115).

Both floral and vegetative characters provided important information about the nature of the plants included in this study. The phenogram based on vegetative characters was more highly structured and similar to the phenogram based on the combined data. The phenogram based on floral characters identified fewer but larger groups. This is not unusual on a low taxonomic level. Floral characters compared to vegetative ones tend to increase in taxonomic significance at higher levels in the taxonomic hierarchy (Davis 1978).

Floral characters clearly distinguished representatives of the 'Retorta' group from the rest, although the 'Retorta' group showed close vegetative links with representatives of the 'Leptophylla' group. The comparison of the three phenograms showed that the assignment of the OTUs 4X/ff and 15A/fc to the 'Fulvida' cluster was based mainly on floral characters. Representatives of the 'Leptophylla' group and coastal populations of the 'Fulvida' group could not be distinguished readily using only floral characters, but they were unlike each other in vegetative characters. In the phenogram based on the combined data the L/F ('Leptophylla/Fulvida') cluster was formed similar to the one based on floral characters, but with the representatives of the 'Leptophylla' group forming their own subcluster. Even if the 'Leptophylla' and the 'Fulvida' cluster showed only small vegetative links, the L/F cluster could be seen as quite stable based on floral and combined data.

2.4.8 Values of the morphological characters used in the study

Character box-plots of macro- and microcharacters included in numerical analysis

Box-plots provided an effective means to allow comparison of character distributions. Analyses were carried out on all quantitative and ordered qualitative characters.
Only a limited number of the characters previously used in keys and descriptions to distinguish taxa within New Zealand's '*Cassinia*' (e.g., Hooker 1853, 1864; Kirk 1899; Cheeseman 1906, 1925; Allan 1961) were supported as useful for distinguishing groups in this study. Indeed, most of these characters were found by Kalin (1967a,b) to be difficult to use for the practical needs of construction of keys and diagnoses because their character states clearly overlap among taxa. From the ranges observed here for characters that have been used in previous descriptions and keys, it was clear that it is not always possible to distinguish the individuals from all 6 groups (defined in Section 2.3.3.2) using single characters such as number of receptacle scales (79), leaf size (24, 25), and leaf shape (20, 21, 22, 23, 26).

The number of receptacle scales (79) clearly distinguished the 'Retorta' group, (with the highest values), and the 'Amoena' group (with no receptacle scales), not just from each other, but also from all other groups (Figure 2.33). The 'Fulvida' group was

separated from the 'Albida' group by a lower number of receptacle scales per capitulum with only some outliers from the 'Fulvida' group overlapping the range of the 'Albida' group. The ranges of scale numbers in the 'Vauvilliersii', 'Fulvida' and 'Leptophylla' groups overlapped quite considerably (Figure 2.33). The scale number varied not only among representatives from different groups, or within one group as seen by the character ranges, but also in individuals and even within the capitula of a single panicle. Such variation can possibly be accounted for by a process in which the scales are split during the ontogeny of the capitulum. The ranges of scale number for the 'Fulvida' and 'Leptophylla' group did not agree with those given in Allan (1961) for '*Cassinia fulvida*' and '*C. leptophylla*', but were very similar to that found by Kalin (1967a).

The ranges for leaf length (24) and width (25) overlapped in a group containing the 'Leptophylla', 'Fulvida', and 'Retorta' groups. The median value for the leaf length in the 'Retorta' group was slightly lower than that of the 'Fulvida' group, but the median value of the leaf width was identical to that of the 'Fulvida' group and slightly higher than that found for the 'Leptophylla' group. The leaf size clearly distinguished the 'Fulvida', 'Leptophylla', and 'Retorta' gropups from the 'Vauvilliersii', 'Albida', and 'Amoena' groups.

Characters used by Allan (1961) to distinguish '*Cassinia amoena*' from the other '*Cassinia* species' such as the absence of receptacle scales (79), a low number of florets per capitula (60), a short growth form (2), and an extremely dense tomentum on the abaxial surface of the leaves (30, 32), were also found in this study to be diagnostic for the 'Amoena' group. Representatives of the 'Amoena' group were also found to have very densely arranged twin hairs (89, MC8) and glandular hairs (90, MC9) on the achenes, which distinguished them from representatives of all other groups. Other characters such as leaf length (24) and capitula size (54, 55, 56, 57) and shape (58, 59) used by Allan (1961) to distinguish '*Cassinia amoena*' did not distinguish the 'Amoena' group from all of the other groups in this study.

By comparing distributions for each character with box-plots, it was also shown that many floral characters permit delimitation of the 'Retorta' group, particularly the length of capitulum (55) and involucre (56), involucre width (56), corolla tube length (61), corolla lobe width (63), diameter of corolla (64), style length (65), stigmatic lobe length (66), outer involucral bract tip colour (68), number of outer involucral bracts (71), inner

involucral bract length (72), number of receptacle scales per capitulum (79), receptacle scale length (80), and pappus length (84).

The 'Retorta' group and the 'Amoena' group were quite different from each other and from the remaining four groups: 'Vauvilliersii', 'Albida', 'Fulvida', and 'Leptophylla'. However, based on vegetative characters, the 'Retorta' group was closer to a group containing the 'Fulvida' and the 'Leptophylla' groups, and the ranges of the vegetative characters of the 'Amoena' group overlapped to a limited extent with that of the 'Albida' and 'Vauvilliersii' groups.

Characters used to distinguish the 'Fulvida' group from the 'Vauvilliersii' group include: a more upright slender growth form with long shoots (1), greater plant height (2), smaller branchlet angle (3) but denser branchlet spacing (10), a mainly acute leaf apex (22), smaller length (24) and width (25) of the leaves, a more strongly recurved margin (27) and a more evident midvein (28) on the leaves, spreading panicles (48), a smaller number of florets per capitulum (60), shorter styles (65), more narrowly ovate outer involucral bracts (67) in smaller numbers (71), shorter (72) and narrower (73) inner involucral bracts, narrower receptacle scales (81), fewer bracts and scales with white tipped appendages (77, 79, 83), and a shorter pappus (84). In all the remaining characters these two groups had identical or largely overlapping ranges.

Characters that may be used to differentiate the 'Fulvida' from the 'Leptophylla' group include: indumentum thickness on branchlets (6), and leaves (29, 30, 31, 32), colour of the abaxial surface of the leaves (15, 17), the main leaf shape (20), the evident midvein (28), the depth of the exudate colour (36, 37), the exudate density on the abaxial surface of the leaves (38), the length of the pedicels (53) and capitula (55), and the number of outer involucral bracts (71). Most of the floral characters had very similar or at least slightly overlapping ranges for the 'Fulvida' and the 'Leptophylla' groups. Median values of some of these characters varied between these two groups (e.g., floret number (60) or number of receptacle scales (79) with higher numbers for both in the 'Leptophylla' group).

The ranges found for the colour and the tomentum density for the 'Albida', 'Leptophylla' and 'Amoena' groups were very similar, and it was mainly in respect to these characters that the 'Albida' group could be distinguished from the 'Vauvilliersii' group.

Clustering based on 15 microcharacters (MC) only (Data Set 7) resulted in a phenogram with unique topology, compared with the results of all previous analyses,

although some clusters showed congruence with those resulting from previous analyses. A breakdown of microcharacter distributions in form of character box-plots was carried out based on the groups supported from analyses of other data, i.e., the 'Vauvilliersii', 'Fulvida', 'Albida', 'Leptophylla', 'Retorta', and 'Amoena' groups. As for the macrocharacters, none of the microcharacters differentiated all 6 groups from each other, but the 'Amoena' group was differentiated from the other groups by wide pappus hairs (MC1, MC4, MC5) with a high number of wide apical pappus cells (MC2, MC3) and long (MC6) and spreading (MC7) pappus barbellae, as well as the highest density of twin hairs on the achenes (MC8, MC9). The ranges of some of the above pappus characters overlapped slightly with that of the 'Vauvilliersii' group. The density of the twin hairs on the achenes (MC8) distinguished not only the 'Amoena' group from the other groups, but also a group consisting of the 'Leptophylla' group and the 'Retorta' group from a group containing the 'Vauvilliersii', 'Albida' and 'Fulvida' groups. Representatives of the 'Albida' group had thick pappus hairs (MC5), which were not much spreading at the tip (MC2, MC2, MC3) and short anther basal appendages (MC14), differentiating this group from the 'Vauvilliersii' group with which it shared most microcharacter ranges. Nearly all microcharacters indicated affinities between the 'Fulvida' group and the 'Leptophylla' group. The 'Retorta' group was only differentiated by the large size of the corolla (MC11) and thus was correlated with the high positioned anther insertion point (MC10).

Despite certain differences of character distributions between different groups, the phenetic structure of *Ozothamnus leptophyllus* relied mainly on polythetic support, i.e., grouping taxa that have the greatest number of shared characters states, with no single character state essential for membership in any particular group (Sneath & Sokal 1973). A similar pattern was found between species of *Sophora* (Heenan et al. 2001), within the *Solanum* series *Longipedicellata* (Spooner et al. 2000), and within the *Linaria depauperata* and *L. supina* complexes (Segarra & Mateu 2001).

External pollen morphology

The external features examined by SEM can often be helpful in suggesting relationships at the lower levels of the taxonomic hierarchy (Stuessy 1990). Pollen morphology and

anatomy has proved to be a useful source of data in the Compositae (Merxmüller et al. 1977, Anderberg 1991). Most of the pollen studies of gnaphalioid Compositae have dealt with relationships at the tribal, subtribal (e.g., Skvarla et al. 1977) and generic level (e.g., Breitwieser & Sampson 1997a). Indication of the potential of pollen morphology as a possible source of information for this investigation of the *Ozothamnus leptophyllus* species complex was given by Breitwieser and Sampson (1997a).

The present investigation of external pollen morphology by means of SEM based on 20 specimens of *Ozothamnus leptophyllus* showed that the pollen is not completely homogeneous. The groups indicated by pollen spine characters were also supported by numerical analysis, based on mainly gross morphological floral and vegetative characters (Section 2.3.3). Pollen Group 1 included OTUs (33B/fc, 65A/fc) described in Section 2.3.3.2 as belonging to the 'Albida' group. Pollen Group 2 included OTUs, which grouped together within the 'Vauvilliersii' clusters of the phenograms based on Data Sets 1-4 (Section 2.3.3.1 and 2.3.3.2). Pollen with more obtuse spine tips within this group belonged to specimens of the 'Vauvilliersii' subgroup 'Vauvilliersii S-Otago/Southland/ Fiordland' (OTU 16A/fc, OTU 17A/fc, and OTU 118B/fc). Pollen of the representative of 'Amoena' also fitted in this group.

The 'intermediate' Pollen Group 3 contained the pollen of OTUs which formed a subcluster of the 'Fulvida' cluster (F) in the phenogram based on Data Set 4 (Section 2.3.3.2). The pollen morphology supports the positioning of OTU 15A/fc and OTU 4X/fc closer to representatives of the 'Fulvida Central Otago/Inland Canterbury' group as seen in the phenogram based on Data Set 4, than to representatives of the 'Vauvilliersii' groups to which they are linked in the phenograms based on Data Sets 1-3 (Section 2.3.3.1). Pollen of the 'Coastal Fulvida' group (OTU 7C/fc and OTU 8G/fc), and of the 'Leptophylla' group (OTU 58B/fc, OTU 70A/fc, and OTU 139B/fc) fell together with the pollen of the 'Retorta' group into Pollen Group 4. All the OTUs with pollen of the Group 4 type have only coastal or lowland distributions.

Breitwieser and Sampson (1997a) showed that the pollen grains could provide characters that are taxonomically useful in classification of New Zealand Gnaphalieae. They included in their pollen studies two forms of *Ozothamnus leptophyllus* (as '*Cassinia fulvida*' and '*C. leptophylla*') and discovered quite remarkable differences in the pollen

morphology between these two forms, i.e., '*C. fulvida*' differed from '*C. leptophylla*' by much shorter spines with a shallow angle and obtuse spine tips. The specimen they called '*C. fulvida*' (WELTU 16016) according to Allan (1961) was collected at Porters Pass Canterbury. In this present study, representatives of populations from the Canterbury mountains like the specimens Breitwieser and Sampson (1997a) included in their pollen studies were assigned to the 'Vauvilliersii' group. The present study did not confirm such a marked difference between different pollen entities, but the relative difference in pollen spine characters between OTUs representing these entities indicated the trend noted by Breitwieser and Sampson (1997a). Pollen grains of representatives of the 'Vauvilliersii' group had shorter spines with a wide base, a shallow angle, concave sides and nearly obtuse tips (Group 2), while pollen with longer spines with a narrow base, steeper gradient, concave sides and subacute tips (Group 4) was characteristic for representatives of the 'Leptophylla' group.

Chapter 3

Molecular studies

3.1 Introduction

Developments in molecular biology, including improvement in methods of DNA isolation, the polymerase chain reaction, and advances in DNA sequencing, have resulted in rapid changes in plant systematics (Soltis et al.1992). Molecular techniques have become of great significance for systematic studies generally and are often seen as an independent source of taxonomic information (Stace 1989, Stuessy 1990). Molecular information, like all other data used by plant systematists, should not be viewed in isolation, but should be compared and contrasted with all other data (Crawford 1990). In addition to providing extensive phylogenetic insights at the highest levels of flowering plants (e.g., Soltis et al. 1999), molecular data may be used to address more narrowly focused questions, including elucidating the relationships for those taxa of uncertain placement due to lack of or conflicting morphological characters (Crawford et al. 2001).

Molecular data have had a significant impact for the phylogenetic reconstruction of the Compositae (Bayer & Starr 1998). DNA sequence data have been used in tribal and intergeneric studies within the Compositae, particularly the Lactuceae (e.g., Koopman et al. 1998), Astereae (e.g., Noyes & Rieseberg 1999, Markos & Baldwin 2001), and Heliantheae (e.g, Kim et al. 1999, Gatt et al. 2000, Clevinger & Panero 2000, Urbatsch et al. 2000). The Phylogeny of South African Gnaphalieae was examined by Bayer et al. (2000) using noncoding chloroplast sequences. Phylogenetic studies of the Gnaphalieae based on nuclear rDNA sequences from the internal transcribed spacer regions (ITS-1 and ITS-2) supported recognition of the genus *Anaphalioides* (Glenny 1997) and estimated relationships in Australasian Gnaphalieae (Breitwieser et al. 1999). Sequencing is likely to continue to provide characters for future analyses of phylogenetic relationships within the Compositae. However, few studies at the intraspecific level have made use of DNA sequence data for a number of reasons, including the difficulty in identifying regions of DNA that vary at the intraspecific level, plus the high cost of sequencing per sample.

DNA fingerprinting techniques have been used for approximately 20 years to generate characters for intraspecific studies. DNA fingerprinting involves the production of a broad sample of an organism's genome. This sample is represented by many fragments

separated according to size by gel electrophoresis and visualised using autoradiography, silver staining or fluorescent dyes. Fingerprinting techniques have been employed not only to study population variation (e.g., Russel et al. 1999), breeding systems (e.g., Krauss & Peakall 1998), gene flow, hybridisation and introgression (e.g., Rieseberg et al. 1999), but also to help to resolve taxonomic and phylogenetic questions (e.g., Kardolus et al. 1998, Aggarwal et al. 1999, Schaal et al. 1998).

Different DNA fingerprinting methods include 1) random amplified polymorphic DNA (RAPD) (Williams et al. 1990); 2) restriction fragment length polymorphism (RFLP) (Tanksley et al. 1989, Francisco-Ortega et al. 1993); 3) microsatellites (Simple Sequence Repeats; SSR) (Schlötterer 1998, Powell et al. 1995, Antonius & Nybom 1994); and 4) amplified fragment length polymorphism (AFLP) (Vos et al. 1995). Such methods are useful for analysis of within-species variation because they allow the rapid acquisition of genetic information representing a large sample of the genome (Hoelzel & Green 1998, Bruford et al. 1998, Harris 1999).

All of these approaches have certain merits but also some inherent disadvantages. RFLP is laborious and results in the detection of a relatively small number of alleles. The detection of RAPD markers is less complex and costly, but is sensitive to reaction conditions and known to have problems of reproducibility (Smith et al. 1994, Williams et al. 1993, Karp et al. 1995). Microsatellites provide high polymorphism but require lengthy studies involving cloning and sequencing in each species to obtain information on flanking nucleotide sequences. AFLP overcomes some of the disadvantages of the earlier techniques and is quickly becoming very popular among agricultural scientists as a versatile and powerful tool in genome analysis.

The AFLP approach was developed by Vos et al. (1995) and is conceptually simple. The procedure draws from both the RFLP and PCR techniques. DNA is cut with restriction enzymes and the resulting millions of fragments are reduced to a hundred or so detectable bands on a gel using two rounds of PCR. Unlike RFLP analysis however, AFLP involves the detection of the presence or absence of restriction fragments rather than differences in their lengths.

In the AFLP procedure, DNA is digested with two enzymes, a rare-cutter (e.g., *Pst* I or *EcoR* I) and a frequent-cutter (*Mse* I); oligonucleotide adapters are then ligated to ends of the fragments. Using commercially produced primers complementary to the ligated adapters, only those fragments with *Pst* I and *Mse* I sites at either end are amplified using

PCR. This initial amplification will select a subset of the fragments which is further reduced to a manageable number through a second round of selective amplification. The second amplification uses primers corresponding to the sequence of the adapter + restriction site bases, plus the first few (one to five) nucleotides of the restriction fragment itself. One extra (selective) nucleotide on each primer will match only one of the four possible nucleotides (A, T, G or C) and thus results in the amplification of only one in sixteen double stranded fragments. The use of two selective bases will decrease the number by 1/256. An average-sized plant genome like soybean amplified with three selective bases on each second round primer will produce about 120 fragments. The practical details for the AFLP techniques have been given in extensive practical reviews like Matthes et al. (1998), and Karp et al. (1995).

The high polymorphism revealed by AFLPs has interested researchers in cultivar/accession identification and population genetics. At present the majority of population genetic uses of AFLPs are for studies of diversity and genetic variation. For example, Russel et al. (1999) investigated the genetic variation of *Calycophyllum spruceanum* (Rubiaceae), Qamaruz-Zaman et al. (1998) showed the use of AFLP fingerprinting in conservation genetics by studying the genetic variation between populations of *Orchis simia* (Orchidaceae), Travis et al. (1996) analysed the genetic variation in *Astragalus cremnophylax* var. *cremnophylax* and Winfield et al. (1998) studied the genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area in the UK. Other studies using AFLP have focused on introgression and hybridisation, for example, Rieseberg et al. (1999) studied introgression between cultivated sunflowers and a sympatric wild sunflower *Helianthus petiolaris* (Asteraceae), Beismann et al. (1997) looked at the distribution of two *Salix* species and their hybrid and O'Hanlon et al. (1999) showed introgression in weedy *Onopordum* thistles. AFLPs have also been used at the level of the individual, for use in paternity analyses and gene-flow investigations, such as Krauss and Peakall (1998), who analysed paternity in natural populations of *Persoonia mollis* (Proteaceae). Aggarwal et al. (1999) investigated the phylogenetic relationships among *Oryza* species using AFLP markers, and Kardolus et al. (1998) applied AFLPs to *Solanum* taxonomy, concluding that AFLPs were "an efficient and reliable technique for evolutionary studies".

The primary reason for the choice of AFLP over alternative methods is that it takes such a large sample of the genome, detecting a high number of DNA fragments or bands,

enabling identification of many polymorphic markers. Routinely about 50-100 bands are observed in each lane of a AFLP gel, compared with about two to five bands obtained in an RFLP analysis, or five to ten bands with the RAPD technique. AFLPs detect more point mutations than RFLPs and are simpler than microsatellites as no prior sequence information is needed. The AFLP does not necessarily offer higher rates of polymorphism but is more efficient in detecting such variation and thus amenable for high throughput screening. AFLPs have broad taxonomic applicability (Mueller & Wolfenbarger 1999) and have been used effectively in the study of a wide variety of organisms, including bacteria (Janssen et al. 1969, Huys et al. 1996), fungi (Majer et al. 1998, Rosendahl & Taylor 1997), animals (nematodes (Semblat et al. 1998), vertebrates (e.g., Giannasi et al. 2001)), cultivated crops (e.g., Maughan et al. 1996), trees (e.g., Beismann et al. 1997, Arens et al. 1998), and ferns (Perrie et al. 2000).

In this part of the study a survey using amplified fragment length polymorphism (AFLP) was conducted to examine genetic diversity in 35 individuals of *Ozothamnus leptophyllus*, of which 12 individuals represented a single homogeneous population. Twenty-three morphologically different homogeneous populations were each represented by one sample. Detection of polymorphism among samples from throughout the country representing the variation found in morphological analysis will provide insight into the level of genetic diversity in the *Ozothamnus leptophyllus* species complex. Two reference taxa from Tasmania were included for comparative purposes (outgroups), *Cassinia aculeata* and *Ozothamnus rodwayi.* They were chosen as they are theoretically closely related to the *Ozothamnus* complex, following the treatment of *Cassinia* and *Ozothamnus* by Breitwieser and Ward (1997), and because fresh cultivated material was available. Estimates of levels of diversity were expected to be greatest between the outgroups and the *O. leptophyllus* complex.

The objectives of this part of the present study are to evaluate the effectiveness of AFLP analysis for detecting genetic diversity in *O. leptophyllus* and to gain an additional set of characters to distinguish between distinct entities of *O. leptophyllus*.

3.1 Material and Methods

3.2.1 Sample collection

3.2.1.1 Representatives from different groups

DNA extractions were made from living plant material that had been maintained in cultivation under uniform conditions for two to 15 months at the University of Canterbury (Section 2.2.1). Material for extraction was taken in April 1999 from leaf buds on young shoots. Samples showing the range of *Ozothamnus leptophyllus* morphological diversity (Section 2.2.1) were collected for analysis from the range of populations shown in Figure 3.1 (see also Table 3.1). Two outgroups from Tasmania were also included, *Cassinia aculeata* and *Ozothamnus rodwayi* (Table 3.1).

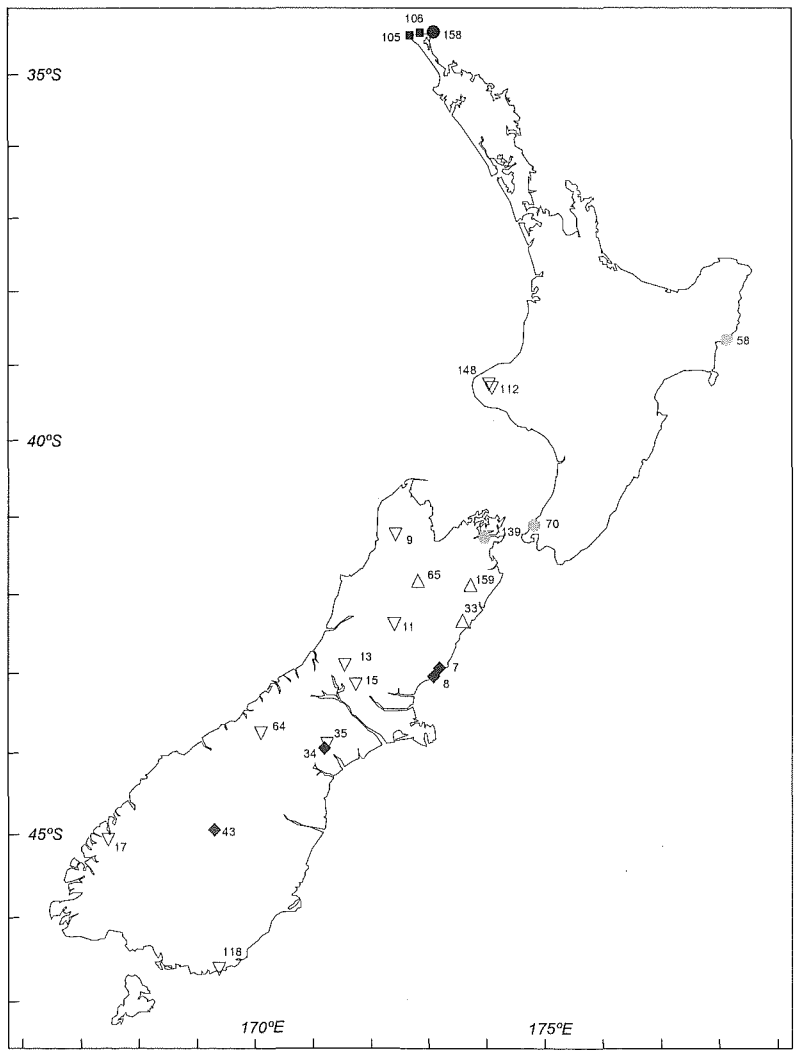


Fig. 3.1 Distribution of the *Ozothamnus leptophyllus* specimens included in the molecular study. ■ 'Retorta', ◍ 'Leptophylla', ◆ 'Fulvida', ▽ 'Vauvilliersii', ● 'Amoena', △ 'Albida.'

Table 3.1 Specimens included in the molecular study.

Number	NZMS 260	Region	Locality	Group	CANU
7 C	N33 194077	N-Canterbury	betw. Napenape and Motunau Beach, Blythe Rd.	'Fulvida Cant. Coast'	38562
8 G	N33 168078	N-Canterbury	Stonyhurst Road (betw. HW1 and Stonyhurst)	'Fulvida Cant. Coast'	38563
9 A	M27 617972	NW Nelson	Garibaldi Ridge, NE facing slope, unnamed peak (1430m)	'Vauvilliersii West Coast'	38564
11 A	M31 606695	Canterbury	W-end of St. James Walk Way, Lewis Pass	'Vauvilliersii var. pallida/albida/canescens' 'Albida'	38565
13 A	K33 913111	Canterbury	Otira Valley, Southern Alps	'Vauvilliersii West Coast'	38566
15 A	K34 074844	Canterbury	upper Cave Stream, between West Coast Rd. and Helicopter Hill	'Vauvilliersii Cant./Otago/N-Southland'	38567
17 A	C42 740575	Fiordland	Lake Wapiti	'Vauvilliersii S-Otago/Southl./Fiordland'	38568
33 B	O31 575745	Marlborough	Mt Fyffe	'Vauvilliersii var. pallida/albida/canescens' 'Albida'	38569
34 A	J37 653952	S-Canterbury	Orari-River Road (Mt Peel)	'Fulvida Central Otago/ inland Cant.'	38570
35 A	J37 685005	S-Canterbury	Peel Forest Park, Deer Spur Track	'Vauvilliersii Cant./Otago/N-Southland'	38571
43 A	G41 176792	Central Otago	Clutha Valley near Crippletown	'Fulvida Central Otago/ inland Cant.'	38572
58 B	Y18 565710	Gisborne	Makorori beach, 10 Min North of Gisborne	'Leptophylla'	38573
64 A	H36 763136	S-Canterbury	Mt Cook National Park: Red Tarn	'Vauvilliersii Cant./Otago/N-Southland'	38574
65 A	N29 943313	Nelson	Mt. Robert Skifield Road (Lake Rotoiti)	'Vauvilliersii var. pallida/albida/canescens	38575
70 B	R27 624094	Wellington	Te Korohiwa Rocks, Titahi Bay	'Leptophylla'	38576
105 A	M02 814497	N-Auckland	Te Werahi Beach, N-Cape	'Retorta'	38577
106 A	N02 979 539	N-Auckland	Hooper Point, N-Cape	'Retorta'	38578
112 A	P20 048 103	Taranaki	Mt Stratford ski field (Mt. Egmont)	'Vauvilliersii N-Island'	38579
118 B	G47 321938	Southland	Cathedral Cave Walk Way	'Vauvilliersii S-Otago/Southl./Fiordland'	38580
139 B	P27 902925	Marlborough	Queen Charlotte Drive, Grove Arm	'Leptophylla'	38581
148 A	P20 003 185	Taranaki	Pouaki Range	'Vauvilliersii N-Island'	38582
158 A	N02 11-55-	N-Auckland	Surville Cliffs	'Amoena'	38583
159 A	P29 933205	Marlborough	Ben More	'Vauvilliersii var. pallida/albida/canescens' 'Albida'	38584
Cass 1-12	K34 089962	Canterbury	population behind Cass Fieldstation	'Vauvilliersii Cant./Otago/N-Southland	
Cassinia aculeata (JW 94115)		Tasmania	Mt Hartz National Park	"outgroup"	
Ozothamnus rodwayi (JW)		Tasmania	Mt Field National Park	"outgroup"	

3.2.1.2 Cass Population

The population of *Ozothamnus leptophyllus* at the Cass field station (NZMS 260 K34 089962) was chosen for study as an example of a homogeneous population. Twelve plants in total were sampled (Section 2.2.1) in June 1999. Specimen material was kept cool for the 2 hr return trip to the laboratory and then maintained at 4°C overnight before extraction.

3.2.2 AFLP Reactions

3.2.2.1 DNA Extraction

All DNAs were extracted from fresh, young leaf tissue following a modified CTAB protocol described in Doyle & Doyle (1987) and Doyle (1991), as this procedure has been reported to provide high quality plant DNA (Milligan 1998): The CTAB buffer (5% w/v CTAB, 1.4 M NaCl, 0.25 M EDTA (ethylene-diaminetetra-acetic acid), 1 M Tris-HCl pH 8.0) and the mortar and pestles were warmed to 65°C, and 0.2% β-mercaptoethanol was added to the CTAB buffer immediately prior to use. Leaf samples were well ground in a mortar with 1 ml CTAB buffer under a fume hood, then transferred into a 2 ml Eppendorf tube. The tubes were then incubated for 30 min at 65°C in a heatblock (Thermolyne DB104320-33). After incubation, 750 µl 24:1 chloroform/isoamyl alcohol was added to each extraction solution and gently mixed by inverting the tubes. The tubes were centrifuged for 4 minutes at 12000 rpm in a Biofuge fresco centrifuge (Heraeus model D-37520). The clear upper phase was pipetted into a clean Eppendorf tube, avoiding the interface. Two-thirds of the volume (approximately 750 µl) ice-cold isopropanol was added and mixed gently by inversion. The tubes were then chilled at -20°C for 2 hours prior to centrifugation for 4 minutes at 12000 rpm to pellet the DNA. The liquid was then carefully poured off, 500 µl 70% chilled ethanol added and the centrifugation repeated. After pouring off the liquid, the pelletted DNAs were air dried by inverting the tubes on a paper towel for 2-3 hours. The pellets were then resuspended in 60 µl TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) buffer.

Agarose gels (30 ml (60 ml) 1x TAE (diluted from 10x TAE containing 48.4 g Tris base (Sigma), 11.42 g glacial acetic acid (Sigma) and 20 ml 0.5 M pH 8.0 EDTA (BDH)) and 0.3 g (0.6 g) Seakem LE agarose) were used to quantify the amount of DNA extracted.

The extracted and resuspended DNA was loaded with a loading buffer alongside a 1kb ladder. The gel was run for 60 minute (40 minutes) at 80 volts and stained after running for 30 minutes with Ethidium Bromide (1mg/l) (Fig. 3.2).

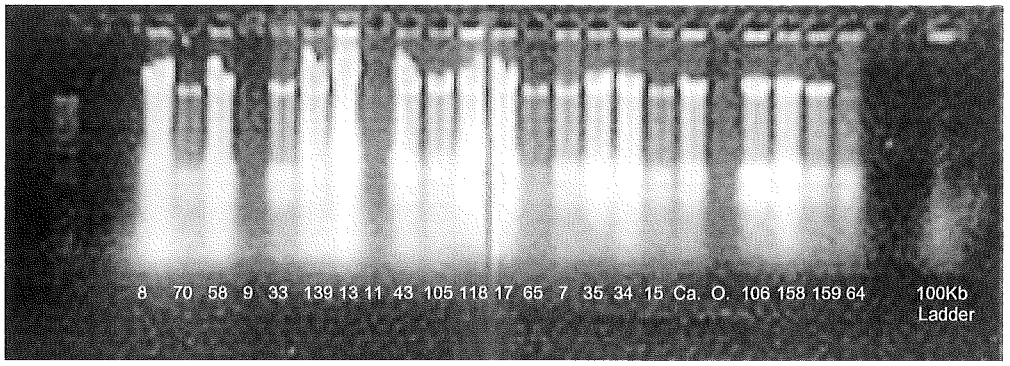


Fig. 3.2 DNA Extractions for some of the 25 samples included in the molecular studies (see Table 3.1 for an explanation of sample numbers and abbreviations) on a 1% agarose gel. Some of the Extractions had to be repeated.

3.2.2.2 Genomic Digestion

A bulk digest cocktail of all components except the genomic DNA was made up for the number of samples plus an additional sample. A total of 40 µl digest cocktail was pipetted into each test tube, and 10 µl DNA was added.

Digest Cocktail per reaction:

volume (µl)	constituents	final concentration
32.75	$_dH_2O$	
5.00	10X OnePhorAll buffer	1x (Pharmacia)
1.25	*Mse* I (4 U/µl)	5 U (Boehringer *Tru* 91)
0.50	*Pst* I (10 U/µl)	5 U (Boehringer)
0.50	BSA (10 µg/µl)	5 µg (NEB)
10.00	50-100 ng/µl genomic DNA	
50.00		

The digests were then incubated at 37°C for 3 hours.

3.2.2.2 Adapter Preparation

Pst I adapter was made from the following ligation recipe:

volume (µl)	constituents
214.50	$_dH_2O$
12.00	10X OnePhorAll buffer
8.10	*Pst* I-1 (1 µg/µl)
5.40	*Pst* I-2 (1 µg/µl)
240.00	

The *Pst* I adapter mix was heated to 95°C in a PCR machine (Hybaid Omnigene) and then allowed to slowly return to room temperature. The final concentration of *Pst* I was 5 pmol/µl. Adapter sequences and annealed forms were as follows:

Adapter Oligo Sequence:

Pst I-1: 5' CTC GTA GAC TGC GTA CAT GCA 3'

Pst I-2: 5' TG TAC GCA GTC TAC 3'

annealed form:

Pst I-1: 5' CTC GTA GAC TGC GTA CAT GCA 3'

Pst I-2: 3' CAT CTG ACG CAT GT 5'

Mse I adapter was made from the following ligation recipe:

volume (µl)	constituents
54.00	$_dH_2O$
6.00	10X OnePhorAll buffer
32.00	*Mse* I Adapter 1 (1 µg/µl)
28.00	*Mse* I Adapter 2 (1 µg/µl)
120.00	

The *Mse* I adapter mix was heated to 95°C in a PCR machine (Hybaid Omnigene), and then allowed to slowly return to room temperature. The final concentration of the *Mse* I adapter mix was 50 pmol/µl. Adapter sequences and annealed forms were as follows:

Adapter Oligo Sequence:

Mse I-1: 5' GAC GAT GAG TCC TGA G 3'

Mse I-2: 5' TAC TCA GGA CTC AT 3'

annealed form:

Mse I-1: 5' GAC GAT GAG TCC TGA G 3'

Mse I-2: 3' TA CTC AGG ACT CAT 5'

3.2.2.4 Ligation of Adapters to Genomic DNA Fragmetns

A bulk ligation cocktail was made up for the number of DNA samples plus an additional sample, and 10 µl was added to each digest tube (Section 3.2.2.2) to a total volume of 60 µl.

Ligation cocktail (per reaction):

volume (µl)	constituents
5.67	$_dH_2O$
1.00	*Pst* I Adapter (5 pmol/µl)
1.00	*Mse* I Adapter (50 pmol/µl)
2.00	T4 DNA ligase 5x buffer (Gibco BRL)
0.33	T4 DNA ligase 3 U/µl (Gibco BRL)
10.00	

This was incubated at 4°C overnight and 5 µl of the ligated digests run on a 1% agarose gel the next day. The gel was stained after running for 30 minutes with Ethidium Bromide (1mg/l) (Fig. 3.3).

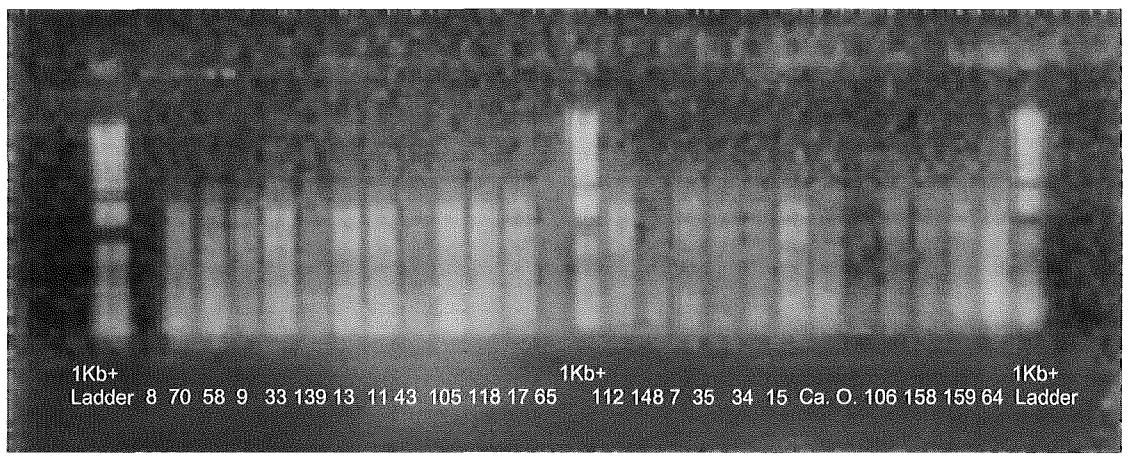


Fig. 3.3 Ligated digest for 25 samples included in the molecular studies (see Table 3.1 for an explanation of sample numbers and abbreviations) on a 1% agarose gel.

3.2.2.5 Pre-Selective PCR amplification (+1N)

5 µl of the ligated digest was diluted 1:5 in sterile TE and used for the pre-amplification round. The remainder was stored at -20°C.

A +1N Amplification bulk cocktail for the number of samples was made up.

Pre-Selective cocktail (per reaction):

volume (µl)	constituents
31.04	$_dH_2O$
2.00	*Taq* DNA polymerase 10x buffer (Boehringer)
1.00	*Pst* I+A primer 50 ng/µl (Gibco BRL)
1.00	*Mse* I+C primer 50 ng/µl (Gibco BRL)
2.00	4 mM dNTP's (Boehringer)
0.16	*Taq* DNA polymerase 5 U/µl (Boehringer)
38.00	

Pre-Selective Primers:

Pst I+N: 5' GAC TGC GTA CAT GCA GN 3' N = A

Mse I+N: 5' GAT GAG TCC TGA GTA AN 3' N = C

38 µl of the cocktail was aliquoted into 200 µl PCR reaction tubes (Life Technologies Australasia) and 2 µl of the diluted genomic digestion/adapter ligation product added.

The samples were run on the pre-selective PCR programme on a MJ Research PTC-200 Peltier Thermal Cycler.

Pre-Selective PCR Programme:

1	94°C	2 min hold	
2	94°C	30 s	⎫
3	50°C	30 s	⎬ 40 cycles
4	72°C	1 min	⎭
5	72°C	5 min	
6	04°C	hold indefinite	

A 5 µl aliquot of each pre-selective product was run on a 1% agarose gel at 80 volts and stained after running for 30 minutes with Ethidium Bromide (1mg/l) to detect faint smears

of amplified DNA (Fig. 3.4). 5 µl of the pre-amplified product was diluted 1:25 in sterile TE and used for the selective amplification round.

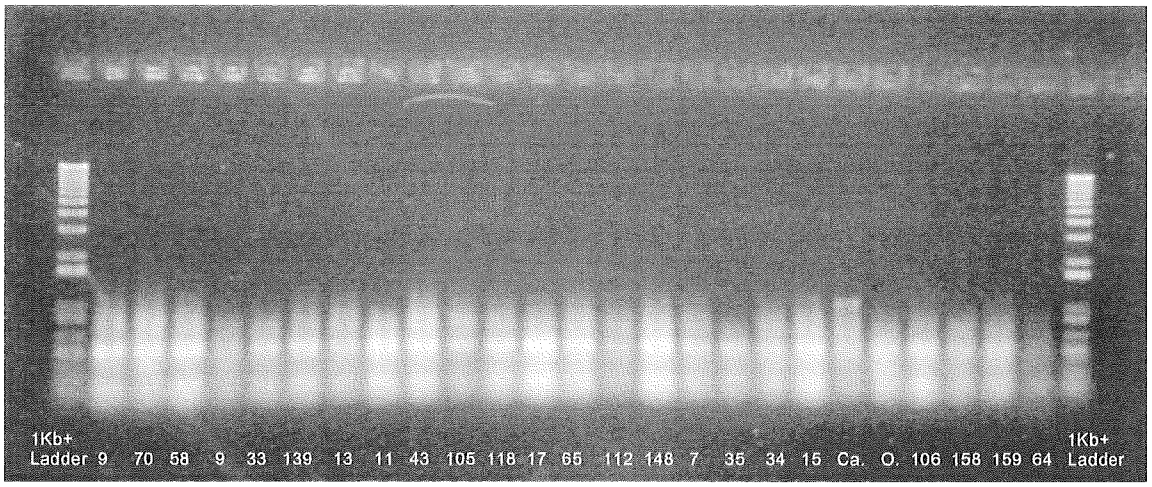


Fig. 3.4 Pre-amplified products for 25 samples included in the molecular studies (see Table 3.1 for an explanation of sample numbers and abbreviations) on a 1% agarose gel. Primer combination *Pst* I+A and *Mse* I+C.

3.2.2.6 Selective PCR amplification (+2N-3N)

A +2-3 N Selective bulk cocktail for the number of samples was made up according to following recipe.

Selective cocktail (per reaction):

volume (µl)	constituents
11.42	$_dH_2O$
2.00	*Taq* DNA polymerase 10x buffer (Boehringer)
0.50	*Pst* I+NN primer 10 ng/µl (Gibco BRL)
0.60	*Mse* I+NN primer 50 ng/µl (Gibco BRL)
0.40	4 mM dNTP's (Boehringer)
0.08	*Taq* DNA polymerase 5 U/µl (Boehringer)
15.00	

Selective Primers were as follows:

Pst I+NN: 5' GAC TGC GTA CAT GCA GN(NN) 3'

Mse I+NN: 5' GAT GAG TCC TGA GTA AN(NN) 3'

(The first N is the same as the N in the pre-selective primers)

15 µl of the cocktail was aliquoted into 200 µl PCR reaction tubes (Life Technologies Australasia) and 5 µl of the 1:25 diluted pre-amplification product added and run in the PCR machine through the AFLP selective programme.

Selective PCR Programme:

1	94°C	2 min hold	
2	94°C	30 s	13 cycles, decrease annealing temperature by 0.7°C each cycle.
3	65°C	30 s	
4	72°C	1 min	
5	94°C	30 s	23 cycles
6	56°C	30 s	
7	72°C	1 min	
8	72°C	2 min hold	
9	04°C	hold indefinite	

The samples were tested by running on a 1% agarose gel at 80 volts which was stained after running for 30 minutes with Ethidium Bromide (1mg/l) (Fig. 3.5).

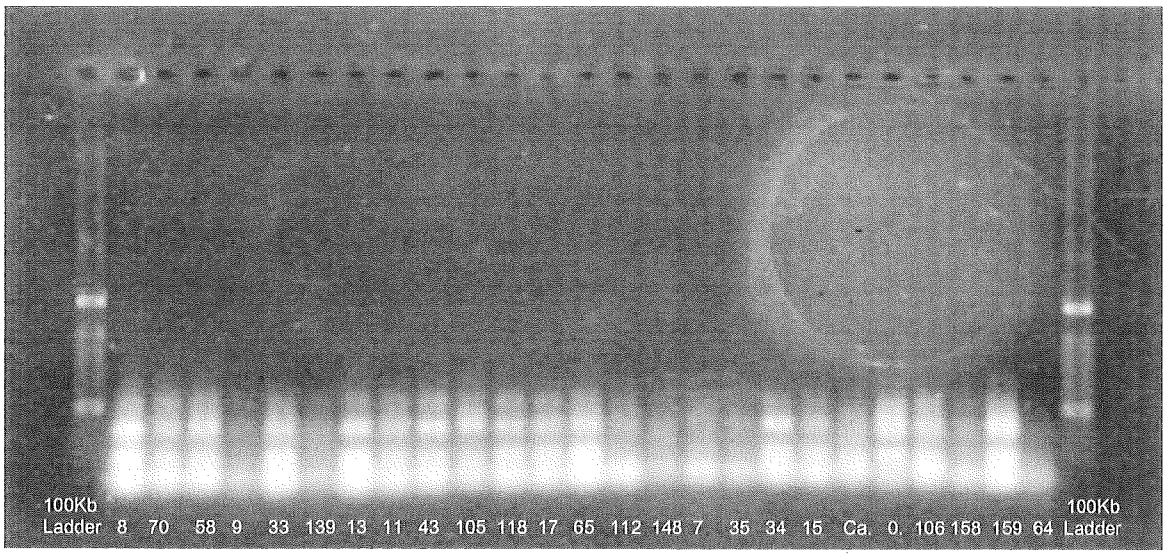


Fig. 3.5 Selective-amplified products for 25 samples included in the molecular studies (see Table 3.1 for an explanation of sample numbers and abbreviations) on a 1% agarose gel. Primer combination *Pst* I+ACT and *Mse* I+CAT.

3.2.3 AFLP Gels

3.2.3.1 Pouring Polyacrylamide Gels

Glass plates were prepared by cleaning with detergent and then rinsing with deionised water. The long plate was treated with 1 ml Rainex Repel-Silane. A total of 100 µl Bind-Silane (2 ml of 95% ethanol, 10 µl acetic acid, 1 µl Bind-Silane (Pharmacia)) was then spread on the short plate, wiped off and then allowed to dry for 4 min. Excess Bind-Silane was removed by 3 washes of 2 ml 95% isopropanol which was then wiped off with a paper towel.

A denaturing urea/polyacrylamide gel was made from the following components:

13.5 ml ReadySol acrylamide (40% acrylamide, acryl:bis=19:1)

9 ml 10x TBE

36 ml $_{d}H_2O$

37.8 g urea

The urea was dissolved in the other ingredients at 65°C. The solution was then cooled to room temperature. Immediately prior to pouring the gel, 450 µl of the accelerator (10% w/v ammonium persulphate) and 45 µl of the catalyst (TEMED) were added and mixed by gently swirling . While the urea was dissolving, the glass plates were taped together with 0.4 mm spacers in place. The acrylamide solution was poured slowly between the plates of glass. The plates were laid down at an angle of approximately 5° to the horizontal and the comb was inserted until the holes in the comb were level with the top of the short plate. Bulldog clips, kept on the spacers only, were used to keep the plates together. Two bulldog clips were placed along the glass over the comb and 2 clips to hold the comb against the long plate. The gel was left to polymerise for one hour.

After polymerisation, any streaks of acrylamide were washed off the outside of the plates, the clips, tape and comb were removed and the wells washed with water. The glass/gel was then inserted into the gel rig (Life Technologies Model S2) with the long plate outermost. The top and bottom tanks were each filled with 500 ml of 1x TBE buffer. The gel was pre run at 55 W (1500 V) for at least 30 min to warm the gel to 55°C.

3.2.3.2 Loading and Running Polyacrylamide Gels

While the gel was pre-running, 4 µl of formamide buffer was added to each sample. Formamide loading buffer:

constituents	quantity	final concentration
Formamide	9.8 ml	98% (v/v)
EDTA (0.5 M, pH 8)	0.2 ml	10 mM
Bromphenol blue	5.0 mg	0.05% (w/v)
Xylene cyanol	5.0 mg	0.05% (w/v)

The samples were then denatured by running in a PCR machine at 94°C for 5 min and then cooled on ice. Before loading the samples, wells were flushed with buffer to remove urea. A spade-tip loading tip (0.4 mm) was used to load 8 µl of each sample. The gel was run at 55°C (55 W, 1500 V) until the remaining dye front (bromphenol blue) was about 4 cm from the base of the gel (4 hours).

3.2.3.3 Staining Polyacrylamide Gels

As soon as the gel run was complete, the upper buffer tank was emptied and the gel with glass plates was removed from the rig. The plates were separated and the plate with the gel was left overnight in a developing tray containing 3 l of 10% (v/v) acetic acid to remove the urea which otherwise interferes with silver staining. After soaking, 2 l of the acetic acid was retained in the freezer for fixing the gel at the end of the staining procedure. The gel was then rinsed 3 times for at least 2 min each time in distilled water to remove the acetic acid. While rinsing the gel the stain solution was made. The stain solution consisted of 3 g silver nitrate, 4.5 ml formaldehyde (37%) in 3 l $_dH_2O$. After the final water rinse, the gel was placed in the Stain Solution and agitated on a shaker for 45 minutes. The developing solution was made by dissolving 120 g sodium carbonate (anhydrous) in 4 l $_dH_2O$ and chilled overnight. Immediately prior to developing the gel, 6.5 ml formaldehyde (37%) and 800 µl sodium thiosulphate (10 mg ml^{-1}) were added to the pre-chilled sodium carbonate solution. The gel was rinsed in chilled $_dH_2O$ after staining, then placed in half the developing solution and agitated. When bands started to appear the gel was transferred to the remaining developing solution which had been stored in the freezer. The bands were developed to their fullest by agitating the tray by hand. Before the gel started to darken, the

reaction was stopped with the chilled acetic acid. The gel was dried overnight, scanned and scored over a light box.

3.2.4 Data Analysis

The presence (1) or absence (0) of the AFLP fragments was scored visually from the gels, and binary matrices prepared for each primer combination. Accurate assessment of shared bands among samples from different gels was facilitated by inclusion of 1kb DNA ladders (Gibco BRL) per gel and by monomorphic fragments. The alignment of fragments that were rarely present (present in only a few samples) was difficult, and uncertain fragments were excluded.

AFLP fragment homology for the New Zealand representatives of *Ozothamnus* was assumed on the basis of the close relationship and overall similarity of band patterns and intensities. There is some additional support for making the assumption of homology among AFLP fragments, especially among closely related taxa (Waugh et al. 1997, Wong & Sun 1999). Homology of fragments of the two "outgroups" *O. rodwayi* and *Cassinia aculeata* from Tasmania was more ambiguous, especially since band patterns and intensities differed.

3.2.4.1 Tests for repeatability

In order to make an estimate of the error associated with the repeatability of the AFLP analysis, two leaf buds from different shoots of the same plant were used for separate extractions from each of the 12 specimens from the Cass population. These samples were carried through the complete AFLP process from extraction through to visualisation of fragments, for all seven primer combinations used in this study.

3.2.4.2 Examination of similarity between primer combinations

Using the numerical taxonomy program NTSYS-pc (Rohlf 1997), similarity matrices were generated from each of the binary matrices for each primer combination based on the Jaccard's association coefficient (S_J) (Sneath & Sokal 1973) (Section 2.2.3.1). The product-moment correlation, r, was calculated between the elements of each of these

similarity matrices, and the Mantel test (Mantel 1967) (Section 2.2.3.4) used to test for significant similarities between primer combinations.

3.2.4.3 Analysis of the complete matrix

The AFLP data from all samples were analysed with descriptive methods and phenetic interpretation. Binary matrices for six primer combinations (excluding primer combination 2: *Pst* I + AC and *Mse* I + CA) were combined to form a single matrix (AFLP Data Set 1). Using the statistical clustering program MVSP Plus Version 3.0 (Kovach 1998) the coefficient of Jaccard was used to generate a matrix of similarities based on the total data (AFLP Data Set 1) for cluster analysis. Jaccard's similarity coefficient ignores negative matches (Section 2.2.3.1), which seems appropriate in the case of AFLP markers (Law et al. 1997) where absence of a band can be the result of different phenomena at the DNA sequence level. Shared absences are over-represented in AFLP data, and more likely to be nonhomologous than shared presences (Wolfe & Liston 1998).

A UPGMA phenogram (Section 2.2.3.2) was constructed and the cophenetic correlation coefficient (Section 2.2.3.4) calculated using the routine "phen.ana5" (Wilton 1999) in S-Plus 4.5 (r2) (Statistical Sciences 1998).

Cassinia aculeata, *Ozothamnus rodwayi*, and the individuals from the Cass population were then excluded from further exploration, which included a second UPGMA analysis and principal coordinate analysis (PCO) (Section 2.2.3.3). The second UPGMA phenogram based on the reduced data set (AFLP Data Set 2) was constructed, the cophenetic correlation coefficient calculated, and the first three coordinates from the PCO plotted. PCO analysis was carried out using MVSP Plus Version 3.0 (Kovach 1998).

3.3 Results

3.3.1 Tests for repeatability

No scorable variability was detected using any of the seven primer combinations between replicate samples included in this analysis (Appendix 12 Figs. 2, 4, 6, 8, 10, 12, 14).

3.3.2 Examination of variability for data from individual primer combinations

A total of 547 AFLP fragments were recorded for 37 OTU's for the seven AFLP primer combinations, with an average polymorphism of 91.6% (Table 3.2).

Table 3.2 Polymorphism detected with 7 amplified fragment length polymorphism (AFLP) primer combinations for 35 *Ozothamnus leptophyllus* samples plus *O. rodwayi* and *Cassinia aculeate*, for 23 *O. leptophyllus* samples without the representatives of the Cass population ( ) and for 12 samples representing the Cass population [ ].

Primer combination			Number of fragments	Number of polymorphic fragments	Percent polymorphism %	Character numbers in complete matrix
No.	*Pst* I	*Mse* I				
1	ACC	CAG	48 (44) [24]	43 (37) [15]	89.6 (84.1) [62.5]	1-48
2	AC	CA	70 (53) [47]	54 (27) [0]	77.1 (50.9) [0]	excluded
3	ACT	CAT	86 (66) [33]	84 (55) [13]	97.7 (83.3) [39.4]	49-134
4	ACG	CAC	79 (61) [31]	74 (50) [9]	92.6 (82) [29]	135-213
5	ACA	CAA	54 (44) [21]	50 (34) [8]	92 (77.3) [38.1]	214-267
6	ACG	CAG	125 (72) [32]	119 (64) [7]	95.2 (88.9) [21.9]	268-392
7	AC	CAG	85 (62) [39]	77 (41) [13]	90.6 (66.1) [33.3]	393-477
Total including No. 2			547 (402) [227]	501 (308) [65]	91.6 (76.6) [32]	
Total excluding No. 2			477 (349) [180]	447 (281) [65]	93 (80.2) [37]	

The primer combinations containing primers with a three base pair extension each (primer combination 1, 3, 4, 5, and 6) revealed some very intensive bands shared by all the samples of New Zealand *Ozothamnus* but less intensive or absent within the "outgroups". These intensive bands are probably related to the amplification of repetitive sequences (Roldán-Ruiz et al. 2000).

Primer combinations varied in their ability to detect polymorphisms, ranging from 77.1% with the primer combination 2 (*Pst* I + AC and *Mse* I + CA) to 97.7% with the primer combination 3 (*Pst* I + ACT and *Mse* I + CAT) for the entire range of samples and 50.9% with the primer combination 2 (*Pst* I + AC and *Mse* I + CA) to 88.9% with the primer combination 6 (*Pst* I + ACG and *Mse* I + CAG) in the reduced data set (Table 3.2).

The primer combination containing primers with a two base pair extension each (primer combination 2), amplified too many low intensity bands for an accurate scoring of the gels. The difference in electrophoretic mobility between bands was very small, increasing the risk for misalignment of the bands. The low number of scored bands for the primer combination *Pst* I + AC and *Mse* I + CA (Table 3.2) is due to the exclusion of uncertain fragments. The fragments obtained from primer combinations 1, 3, 4, 5, 6 and 7 are likely to be, at least partial, subsets of those fragments obtained through amplification using primer combination 2. Fragments obtained using primer combination 2 were therefore excluded from multivariate analyses to avoid logical correlation between characters.

The correlation coefficients calculated between primer matrices using the Mantel test ranged between $r = 0.56$ and $r = 0.82$ (Table 3.3). The correlation of the similarity coefficients was significantly different from zero at the 0.1 percent level ($p < 0.001$). Therefore the correlations between primer matrices were highly significant.

Table 3.3 Matrix correlation statistics, r (= normalised Mantel statistic Z), for six amplified fragment length polymorphism (AFLP) primer combinations. Results of the Mantel test were highly significant between primer matrices, with $p = <0.001$ for all six combinations.

	Primer Combination						
Primer Combination		1	3	4	5	6	7
	1		0.59	0.58	0.56	0.61	0.62
	3			0.74	0.70	0.73	0.74
	4				0.65	0.80	0.78
	5					0.71	0.66
	6						0.71
	7						

3.3.3 Analysis of the complete matrix

Reference taxa ("outgroups") and members of the single population were clearly distinguished using UPGMA clustering (Fig. 3.6). *Cassinia aculeata* and *Ozothamnus rodwayi* were separated from other OTUs, and the members of the Cass population grouped closely together at a high level of similarity (0.853), with Jaccard similarities among the AFLPs of individual plants ranging between 0.859 and 0.931 (Appendix 8).

O. rodwayi joined the phenogram at a similarity level of 0.411 and *C. aculeata* was even more distantly linked at 0.259. The representative of the 'Amoena' group, OTU 158A was in an isolated position joining at 0.510. The cluster containing members of the Cass population joined the phenogram at 0.604. The OTUs representing the different groups formed a multiple structured cluster in which, above a similarity level of 0.692, four main clusters occurred.

The overall cophenetic correlation coefficient of the UPGMA phenogram based on Data Set 1 of the molecular data (Fig. 3.6) was 0.97653 (Pearson) and 0.82345 (Spearman). Dramatic drops (down to 0.65) of the correlation coefficient (Pearson) occurred when members of the Cass population linked at high levels of similarity (Fig. 3.8). After all members of the Cass population had been added to the cluster, the

cophenetic correlation coefficient stabilised at a relatively high value between 0.87 and 0.98. The cophenetic correlation plot (Fig. 3.8) showed that the correlation between the similarity matrix and the phenogram is always relatively high except when some representatives of the Cass population are joined together.

Following the exclusion of *C. aculeata*, *O. rodwayi*, and the Cass population, UPGMA clustering based on AFLP Data Set 2 allowed good separation of OTUs representing different groups (Fig. 3.7). The overall cophenetic correlation coefficient was 0.91749 (Pearson) and 0.8675 (Spearman). Four main clusters could be distinguished and the representative of the 'Amoena' group (A), OTU 158A, joined distantly at 0.541. Cluster designations were as follows: ALB = 'Albida' (representatives of the 'Albida' group), V = 'Vauvilliersii' (representatives of the 'Vauvilliersii' group), R = 'Retorta' (representatives of the 'Retorta' group), and L/F = 'Leptophylla/Fulvida' (representatives of the 'Leptophylla' group and the 'Fulvida' group) (as for previously described phenograms based on morphological data, Section 2.2.3).

Cluster ALB and V linked together at a similarity level of 0.664 and R and L/F linked at 0.639. Cluster ALB, V, R, and L/F linked at 0.611.

Cluster ALB is composed of OTU 65A forming a pair with OTU 159 at 0.803, which is joined at 0.710 by OTU 33B. Cluster V is split into two clusters, which join at 0.692. Within the first of these two V-subclusters OTU 118B and OTU 17A (both representatives of the 'Vauvilliersii S-Otago/Southland/Fiordland' group), form a pair (at 0.792) and linked together at 0.714 with an other pair, formed by a specimen from Mt Cook (OTU 64A) joined with one from the Canterbury Ranges (OTU 15A) at 0.756. In the second V-subcluster two North Island representatives of the 'Vauvilliersii' group (OTU 112A and OTU 148A) linked together at 0.801, before joining at 0.700 a cluster which was composed of a pair formed by OTU 9A and OTU 13A, two representatives of the 'Vauvilliersii West Coast' group, linked at 0.764 and an other pair formed by OTU 11A (originally assigned to the 'Vauvilliersii var. pallida/albida/canescens' group) and OTU 35A ('Vauvilliersii Cant./Otago/N-Southland' group) linked at 0.805.

The R cluster contains the two representatives of the 'Retorta' group included in the molecular study, closely linked at 0.919. The L/F cluster is composed of lesser clusters L and F, which joined at 0.716. Cluster L comprises of South Island (OTU 139B) and two North Island (OTU 58B and OTU 70B) specimens from the 'Leptophylla' group. OTU 139B and OTU 70B linked together at 0.835, while OTU 58B joined more distantly (at

0.804). Cluster F consists of OTUs from the 'Fulvida' group. Two specimens of the 'Fulvida Central Otago/inland Canterbury' group, OTU 34A and OTU 43A, form a pair at 0.868 and linked together at 0.780 with an other pair formed by representatives of the 'Fulvida Canterbury Coast' group (OTU 8G and OTU 7C at 0.858).

The cophenetic correlation plot (Fig. 3.9) shows that the correlation between the similarity matrix and the phenogram is always relatively high (between 0.85 and 1.0). No dramatic drop of the correlation coefficient occurs. The cophenetic correlation coefficient (Pearson) for the average linkage phenogram based on the reduced molecular data set (Fig. 3.9) dropped slightly to 0.95 when the OTU pair 11A and 35A linked with another 'Vauvilliersii' pair: OTU 9A and OTU 13A at 0.764. The correlation coefficient then stabilised at 0.95 for the links occurring between 0.716 and 0.710, and dropped subsequently to 0.87 at 0.692 where all the OTUs of cluster V link together. A further drop occurred down to 0.85 occurred at 0.665, where cluster ALB joins the cluster V. From this lowest value the coefficient rises again up to 0.92 for the links occurring between 0.639 and 0.541.

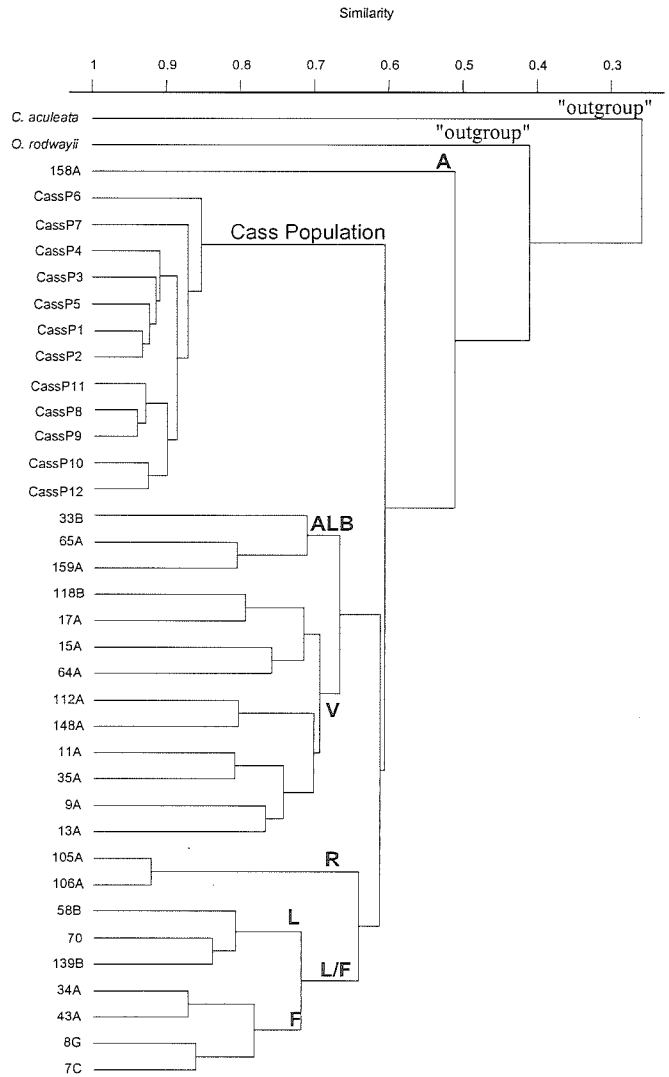


Fig. 3.7 UPGMA phenogram based on AFLP Data Set 1 (including *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population). Abbreviations are as given in the text.

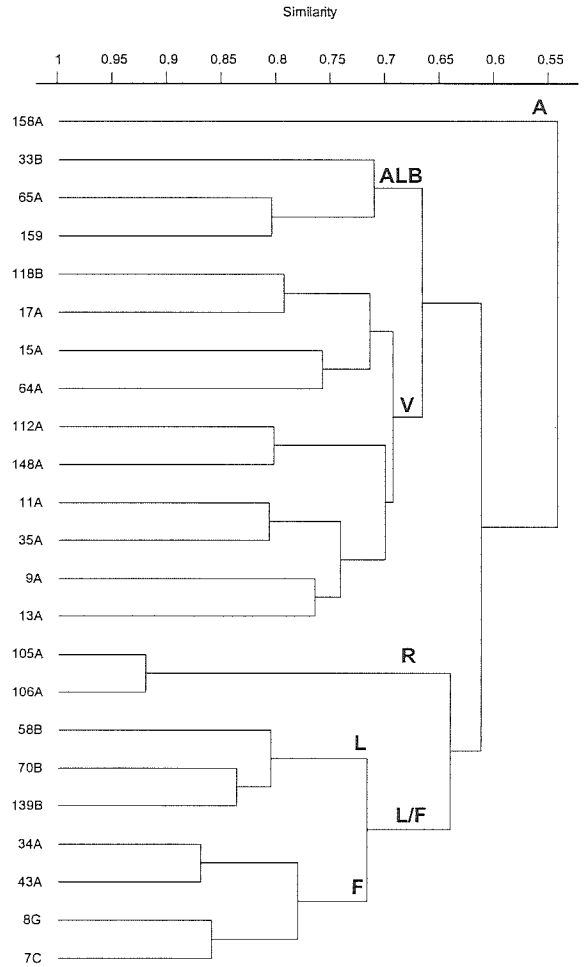


Fig. 3.7 UPGMA phenogram based on AFLP Data Set 2 (without *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population). Abbreviations are as given in the text.

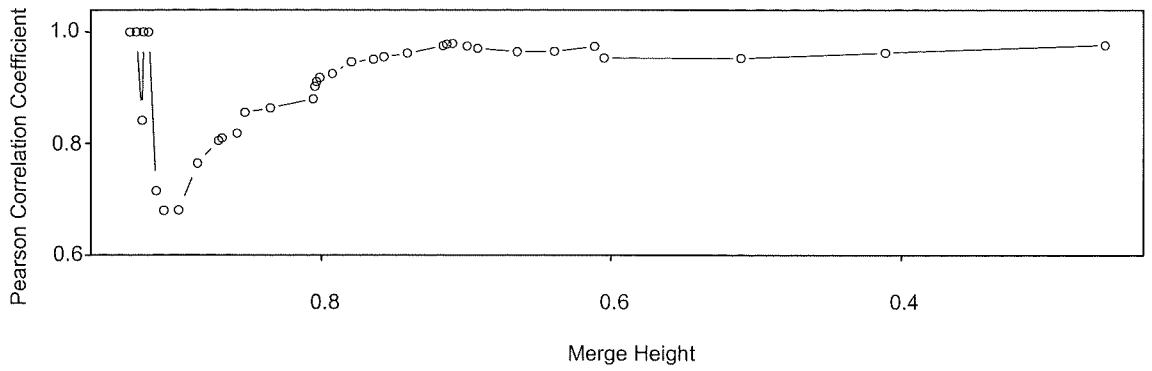


Fig. 3.8 Plot showing the change in the cophenetic correlation coefficient (Pearson) as taxa are clustered in the UPGMA phenogram based on the AFLP Data Set 1(including *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population); overall cophenetic correlation value: 0.976.

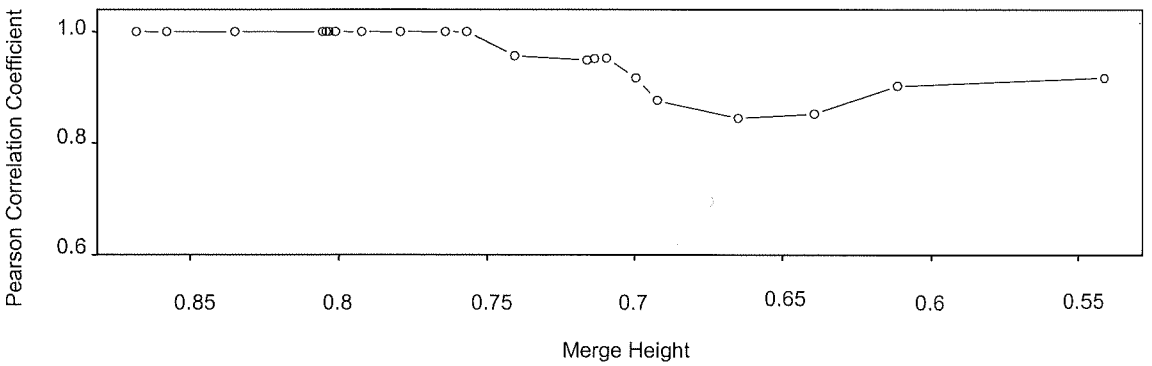


Fig. 3.9 Plot showing the change in the cophenetic correlation coefficient (Pearson) as taxa are clustered in the UPGMA phenogram based on the AFLP Data Set 2 (without *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population); overall cophenetic correlation value: 0.917.

The four clusters ALB, V, R, and L/F (composed of the L and F clusters) as well as the representative of the 'Amoena' group (A), in the UPGMA phenogram based on the reduced molecular data set (AFLP Data Set 2) were also reflected in the PCO plots. The first coordinate explained 17.5% and the second and third coordinates explained 9.9%, and 8.7% of the total variation, respectively. Principal coordinate axes 1 and 2, 1 and 3, and 2 and 3 are shown in Fig. 3.10-3.12. Principal coordinate 1 primarily distinguished two

consisting of 'Vauvilliersii', 'Albida' and 'Amoena' groups, the other composed of the 'Leptophylla/Fulvida' group. The 'Retorta' group is located between these two groups. Principal coordinate 2 primarily separated a single group of two OTUs representing the 'Retorta' group, but also showed a small degree of separation of the 'Leptophylla' group from the 'Fulvida' group, and of the 'Albida' group from the 'Vauvilliersii' group. Principal coordinate 3 separated the 'Fulvida' from the 'Leptophylla' group and split the 'Vauvilliersii' group away from the 'Albida' group, which shows some affinity with 'Amoena'.

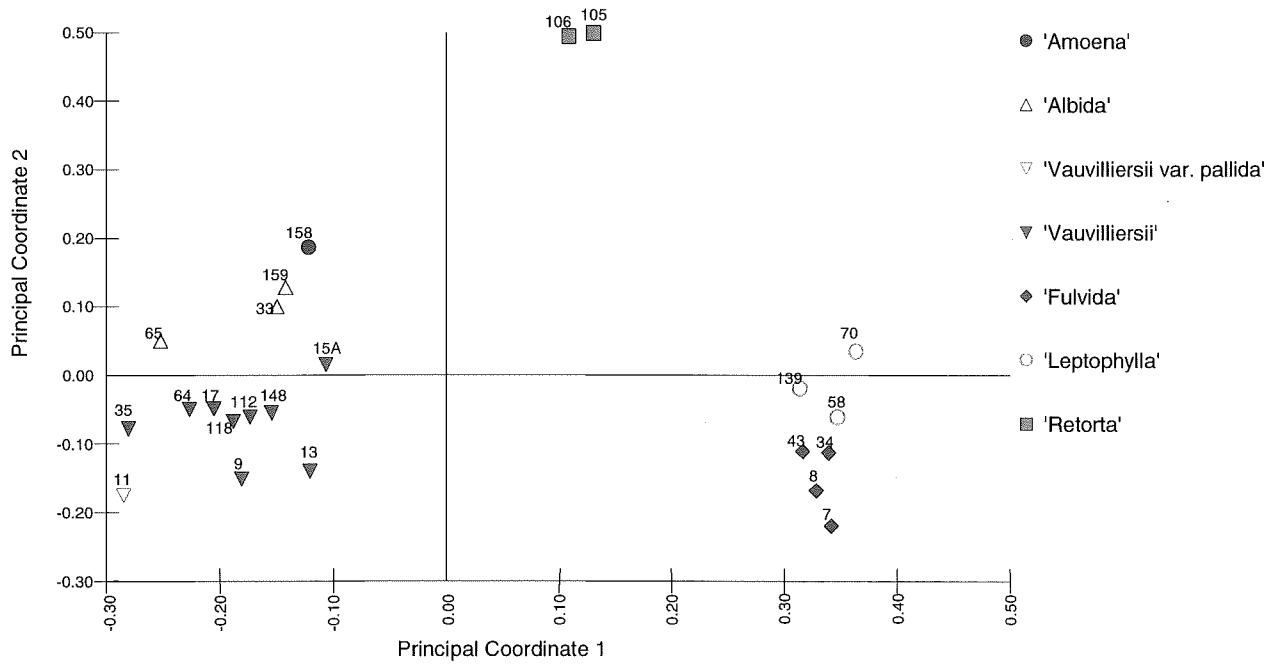


Fig. 3.10 Plot of principal coordinate analysis 2 vs. 1 from the AFLP Data Set 2 (without *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population).

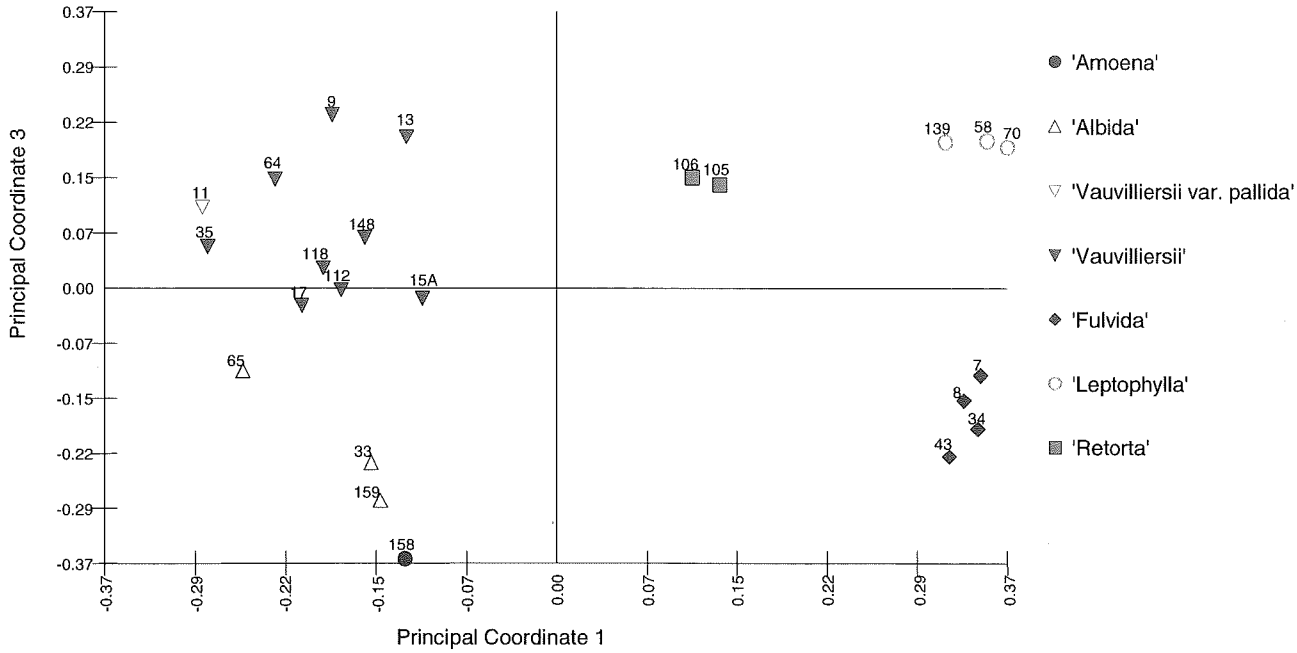


Fig. 3.11 Plot of principal coordinate analysis 3 vs. 1 from the AFLP Data Set 2 (without *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population).

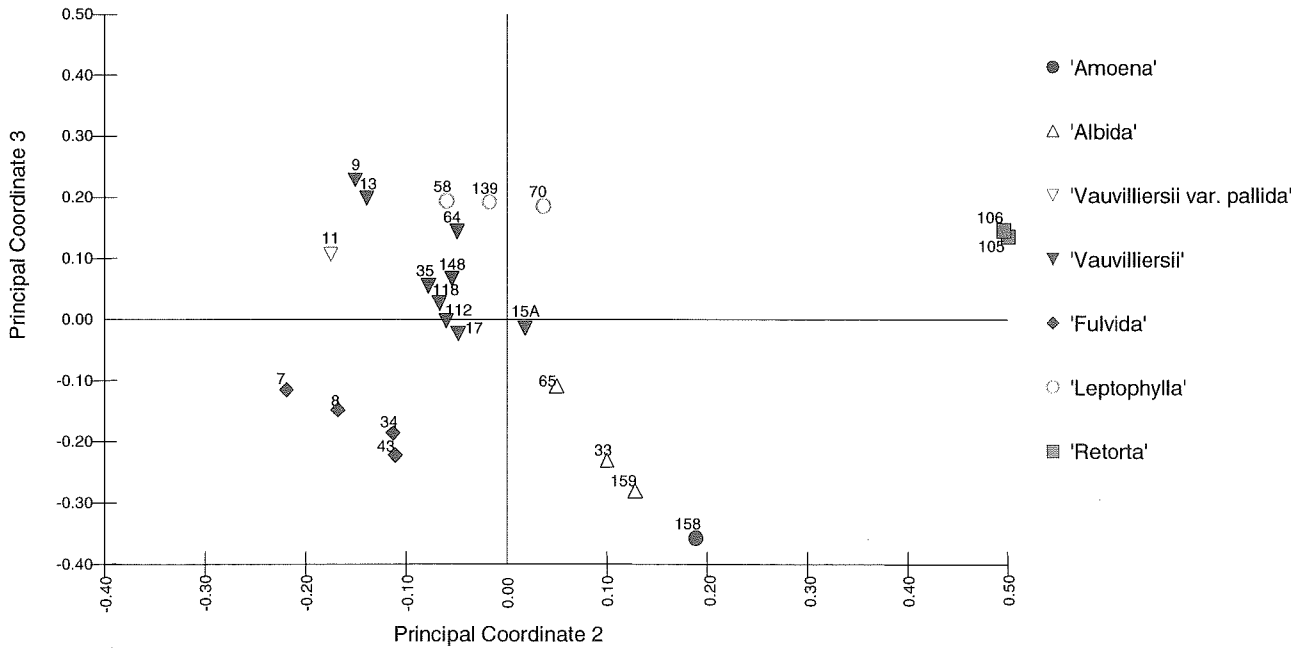


Fig. 3.12 Plot of principal coordinate analysis 3 vs. 2 from the AFLP Data Set 2 (without *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population).

3.4 Discussion

The results demonstrate the usefulness of the AFLP procedure for obtaining information on levels of genetic diversity within and among populations of *Ozothamnus leptophyllus*. As a DNA fingerprinting technique, AFLP is proficient in revealing diversity at and below the species level, providing an effective means of covering a large portion of the genome in a single assay. Nevertheless there are possible ways by which the variation detected may deviate from the true level of variation between individuals.

Two different events may lead to appearance of an amplified fragment of the same size. Since the number of fragments amplified is so high, there is the possibility that two different fragments have the same size. Thus, two polymorphisms would be scored as one change only, leading to an underestimation of variation. Hill et al. (1996) compared the genetic relationship obtained by restriction fragment length polymorphism (RFLP) and AFLP markers in *Lactuca* spp. and found a strong correlation, which indicates that the problem is not common or does not affect results. Another study by Lu et al. (1996) showed that results obtained in pea were not different between methods (cDNA-RFLP, random amplified polymorphic DNA, microsatellites and AFLP).

The loss of restriction sites, insertions or deletions, might also result in an overestimation of variation (Quamarus-Zaman et al. 1998). This is due to the fact that 'fragment' changes rather than 'site' changes are scored. Bremer (1991) suggests that an "overscoring" caused by above events will be randomly distributed and so should not systematically bias the results, especially if groups are strongly supported.

The main disadvantage of AFLP markers is that alleles are not easily recognised (Majer et al. 1996). Allelic fragments will be scored as independent, although in reality they are not. This could lead to an overestimation of variation. AFLPs have to be treated as dominant markers because the identity of homozygotes and heterozygotes cannot be established (unless breeding studies are carried out to determine inheritance patterns for each band). Maughan et al. (1996) has reported putative allelic markers indicating that the data must be looked at carefully and preferably checked genetically. However, the large number of bands gives a measure of variation across the genome, thus providing a good general picture of the level of genetic variation.

Six of the seven primer combinations tested in this work revealed clearly resolved fragment patterns and could therefore be used in future studies to estimate genetic

similarities between *Ozothamnus* plants. The fragments obtained from the primer combination containing primers with a two base extension each (combination 2), were excluded from further analysis. At least some of the fragments from combination 2 could have been duplicated in the profiles generated using primer combinations with three base extensions. There is considerable scope for detecting further polymorphisms through the use of other primer combinations, other restriction enzymes and other adapters. About 93% of fragments amplified in 35 *Ozothamnus* samples plus *O. rodwayi* and *Cassinia aculeata* were polymorphic. 80.2% polymorphism could be recorded for 23 *Ozothamnus* samples representing different groups. This value, compared with 37% polymorphism within the Cass population indicates that there is considerably more variation between populations than within a single population.

A problem using AFLPs may be the redundancy between information provided using different primer pairs (Cresswell et al. 2001). While there have been reports of low (Tohme et al. 1996) and high (Roa et al. 1997) correlations between AFLP primer pairs, the correlation between primer matrices for this study were highly significant, ranging between $r = 0.56$ and $r = 0.82$.

The gels were scored visually. Visual inspection is very accurate when only a few patterns have to be scored and especially when many monomorphic bands are present that can be used as references to align corresponding polymorphic bands, but rather time-consuming and error-prone when large numbers of highly polymorphic fingerprints localised in different gels are analysed (Roldán-Ruiz et al. 1988). In this study it proved to be extremely difficult to compare the band patterns of samples on different gels with each other. Band alignment and gel normalisation are influenced by electrophoretic parameters (Huys & Swings 1999). The samples representing the Cass population and those representing different populations were placed on two different gels for each primer combination and subjected to two different electrophoresis runs, but were scored together and recorded in the same binary matrix. The alignment of fragments that were rarely present was very difficult and even though uncertain fragments were excluded, UPGMA analysis showed that the Cass population was placed quite separate without any obvious close link to any of the other populations. Even if this outcome is not exclusively due to scoring discrepancies but mainly caused by the chosen cluster algorithm (see below), difficulties in aligning the fragments from two different gels might have added to the

positioning of the Cass population cluster and led definitely to a loss of scorable fragments.

Computer-assisted scoring could help to avoid discrepancies caused by difficulties to align fragments of samples which were subjected to different electrophoresis runs. Even so, this involves a series of post-electrophoresis steps, such as autoradiography followed by digitisation of imaging and/or (semi) automatic corrections for gel deformations (Roldán-Ruiz et al. 2000). Using fluorescent detection, the process of producing and scoring the fluorescent fingerprints could be completed far more accurately and efficiently (Krauss & Peakall 1998). Several recent studies (e.g., Huys & Swings 1999, Roldán-Ruiz et al. 2000) showed that the use of a fluorescent detection approach could contribute greatly to the speed and ease of conducting and interpreting AFLP patterns. Fluorescent AFLP methods were not used in the present study due primarily to the ready availability of equipment for polyacrylamide electrophoresis and silver staining in our laboratory. Also the general opinion at this time was that fluorescent AFLP methods would be more expensive. In fact these methods today are cheaper and easier (Huys & Swings 1999, Roldán-Ruiz et al. 2000) and future research of this type is likely to utilise fluorescent techniques.

AFLPs are generally acclaimed for their reproducibility, which sets the technique apart from RAPDs. AFLP fingerprinting, in contrast to RAPDs, is insensitive to variation among reaction mixtures, such as template concentration (Vos et al. 1995). Primer annealing is very specific because primers are homologous to both the adapter sequence and the restriction site sequence. Anomalies can arise if the template DNA is only partly digested, which is avoided by providing adequate digestion time and digestive enzyme concentration relative to template present.

A high reproducibility of AFLP data could be shown in this study. The duplicates returned identical banding patterns. The only differences observed among reactions in the test for repeatability were in the intensity of the bands. This agrees with earlier estimates by Huys et al. (1996), of 95-98.5% correlations of reference samples, when AFLP reproducibility in *Aeromonas* was assessed using a single strain as an internal reference during each electrophoresis run. Similarly, Hongtrakul et al. (1997) found no discrepancies when comparing duplicate AFLP patterns of sunflower inbred lines and Winfield et al. (1998) encountered similarities of 93-100% between duplicated samples of *Populus nigra* subsp. *betulifolia*.

In order to provide a visual representation of the genetic distinction of putative distinct taxa, UPGMA clustering and PCO were used. UPGMA analysis, carried out for both of the molecular data sets provided a good fit to the data, as reflected by a high overall cophenetic correlation coefficient for both phenograms.

From UPGMA analysis of the entire molecular data set (AFLP Data Set 1, Fig. 3.6), which included the Cass population and the outgroups, it was evident that genetic differentiation within the Cass population is less than that among most populations. Without exception, all plants within the Cass population branched from a single population-specific node and linked with each other on a high level of similarity. The clustering within the Cass samples is characterised by a poor fit to the data, as reflected by low cophenetic correlation values (Fig. 3.8). This is not surprising considering the Jaccard coefficients between the representatives are very similar and often identical, which can cause problems in calculating a phenogram (Sneath & Sokal 1973).

The positioning of the Cass cluster must be seen as an artefact caused, besides scoring difficulties (see above), mainly by the way clusters are formed using the unweighted pair-group method using arithmetic averages (UPGMA). The distance between clusters is the average distance between all possible pairs of members of the two clusters (Section 2.2.3.2). UPGMA is sensitive to cluster size, and a distortion occurred due to the uneven sample sizes for different groups. A comparison with the similarity matrix (Appendix 8) easily revealed this distortion. The highest similarity values (ranging between 0.65 and 0.69, with an average of 0.67) could be found between the representatives of the Cass population and OTU 15A, a representative of the 'Vauvilliersii Canterbury/Otago/N-Southland' group, the same group the Cass population was assigned to. High similarity values are also present among most of the representatives of the Cass population and representatives of some other 'Vauvilliersii' representatives (OTU 17A, OTU 148A, OTU 13A), and OTU 7C of the 'Fulvida' group.

The positioning of the two outgroup taxa in phenetic analysis using AFLP data shows that *Ozothamnus rodwayi* is more similar to New Zealand specimens of *Ozothamnus leptophyllus* than is *Cassinia aculeata*. Both outgroups were clearly separated from the remaining individuals, giving an impression of genetic diversity between a Tasmanian *Ozothamnus*, a Tasmanian *Cassinia* and the New Zealand specimens of *Ozothamnus leptophyllus*.

UPGMA analysis as well as PCO analysis of the reduced molecular data set (AFLP

Data Set 2, Fig. 3.7, and Fig. 3.10-3.12) showed that different morphological and geographical groups of *O. leptophyllus* are well reflected in the AFLP pattern. Five distinct groups could be distinguished. The 'Amoena' group, represented by one specimen only, was clearly separated in the UPGMA phenogram from the other specimens of *Ozothamnus leptophyllus* included in this study. PCO analysis revealed 'Amoena' had affinities with the representatives of the 'Albida' group. These findings correspond with the previous classification based on morphology, and with the results of the numerical analysis based on morphological characters in this study (Chapter 2). The 'Albida' and 'Vauvilliersii' groups are linked together in the UPGMA phenogram. Within both groups the level of genetic diversity is quite high. Representatives of geographically and morphologically close populations within the 'Vauvilliersii' cluster seem to be less genetically distinct and form subclusters. The level of genetic diversity within these subclusters is higher than that within the Cass population. The two 'Retorta' specimens are linked at a similarity level comparable to that at which representatives of the Cass population join each other. The 'Leptophylla/Fulvida' group comprises two subgroups, which are less genetically distinct than some of the subgroups within the 'Vauvilliersii' cluster if only the UPGMA phenogram is considered. Principal coordinate 3, explaining 8.7% of the variation separates these two subgroups clearly from each other.

The results of the AFLP data analysis show good resolution among individuals and reasonably high vector percentages in the PCO analysis. This can be interpreted as low levels of genetic exchange among most of the individuals included (Vijverberg 2001) and a reasonably high level of overall genetic distance within the distinct forms of New Zealand *Ozothamnus*. This might have been quite different within heterogenous populations and if intermediate forms had been included in the molecular study. However, the aim of this study was to test whether there are genetically distinct forms within the *Ozothamnus* species complex and whether these forms correspond with previous taxonomic units as well as with the results of morphological analysis presented in this thesis. Other researchers (e.g., Hill et al. 1996, Lu et al. 1996) have also found that AFLP analysis is an efficient and reliable technique to generate data for fine taxonomic levels.

In summary, reliable biosystematic data for evaluation of distinctiveness of taxa may be produced rapidly using the AFLP technique.

Chapter 4

Comparison of morphological and molecular analyses

4.1 Introduction

Controversies over the relative value of different types of systematic data have appeared periodically (e.g., Humphries 1988, Soltis et al. 1992, Lamboy 1994), but most systematists recognise that both morphological and molecular data each have distinct advantages (e.g., Hillis 1987, Patterson 1987, Mishler 2000). For example, morphological techniques are applicable to an enormous range of museum and fossil material. Morphological characters can be analysed quickly and cheaply in large numbers of individuals, and find practical use in keys and descriptions (refer also to Section 2.1). On the other hand, molecular characters have a clear genetic basis and are limited only by the genome size. Molecular data have contributed most significantly in areas where morphological data are inconclusive or deficient (e.g., Patterson et al. 1993, Rieseberg et al. 1996, Loh et al. 2000, Triest 2001).

It is important to select methods of analysis that are as free as possible of assumptions and are amenable to the comparison and/or combination of different types of data. The numerical technique using similarity measures has frequently been applied to analyse character sets based on both morphology and AFLP data (e.g., Chiapella 2000, Perrie et al. 2000, Evens et al. 2002).

A meaningful combined analysis of the two is not possible if, as in this study, AFLPs are carried out using non-specific primers. Using AFLP analysis, unknown parts of the genome are amplified and it is not possible to draw conclusions about specific regions of the genome. The present paucity of knowledge regarding which genes determine which morphological traits makes a combined analysis of the morphological and AFLP data inappropriate because the AFLP fragments cannot be treated as characters of the same weight as the morphological characters. The best agreement between morphological characters and AFLP markers is probably attained when using character-specific primers, or when the AFLPs are linked to selected loci (Mishler 2001 pers. comm.). Only in this

case can character matrices from morphological and AFLP analysis be combined and analysed together (Mishler 2001 pers. comm.). With future knowledge about genes controlling morphometric characters, it may be possible to use primers specific for detecting gene specific polymorphisms.

A comparison of the results of the morphological and AFLP analyses is necessary to investigate if distinct morphological forms are also genetically distinct. Congruence between the results of morphological and molecular investigations of systematic problems is strong evidence that the underlying pattern has been discovered. Conflict may indicate theoretical or procedural problems in one or both analyses (Kadereit 1994), or it may point towards the need for additional data to resolve the systematic problem in question (Hillis 1987). For example, Black-Samuelsson et al. (1997) encountered low agreement between morphological and RAPD characters in populations of *Vicia pisiformis*, and suggested the use of trait-specific primers or the linkage of RAPDs to selected loci. Other studies comparing morphological and molecular markers in plants also found considerable disagreements. Vijverberg (2001) observed a lack of a good reflection of the conspicuous morphological variation within New Zealand and Australian *Microseris* in the nuclear genetic structure, using AFLPs. She explained this by the involvement of only a few genes in the ecotype characteristics and/or an inefficient detection of these genes by the AFLP method. The AFLP method approaches a random sampling of mostly neutral nuclear DNA markers (Vos et al. 1995). Due to this it is likely that it might not be sensitive enough to pick up the genes responsible for morphological variation on the lowest taxonomic levels, as in the study of Vijverberg (2001), where the adaptively radiated New Zealand and Australian *Microseris* have been examined.

On the other hand there are several studies in which molecular and morphological analyses show high similarities. Perrie et al. (2000) showed that two morphological forms of the *Polystichum richardii* complex could be genetically distinguished using AFLPs. They proposed the recognition of two distinct species within this complex, based on the molecular and morphological concordance. The AFLP technique and the morphological evaluation of flower and leaf characters led to the same clear discrimination between three *Hibiscus* species (Van Huylenbroeck et al. 2000). Cultivars have also been distinguished using both leaf indices and AFLP markers. A good correlation between the large

morphological and molecular variation in *Hordeum chilense* species was found by Vaz Patto et al. (2001). They concluded that *H. chilense* consists of at least three well-defined, morphologically and genetically distinct subspecies. A study by Evans et al. (2002) supported the current taxonomic classification of the *Banksia integrifolia* species complex by demonstrating a significant degree of congruency between morphological and molecular characters of *B. integrifolia* and *B. aquilonia*. Despite these reports, few plant groups have been well investigated using both morphological and molecular methods, especially at or below species level (Vijverberg 2001).

In the present study, representatives of the *Ozothamnus leptophyllus* complex have been investigated for their morphological variation and, using AFLPs, nDNA variation. Morphological investigations produced evidence for the occurrence of distinct groups within the complex, but also indicated possible hybridisation events among populations of different groups that have produced intermediate forms. Such intermediate forms were excluded from the molecular study. The AFLP data set showed high overall genetic distances between populations and little variation within a homogenous population.

The objective of this part of the study is to compare the results of morphological and molecular analyses in order to discover if there is a concordance between the two sources of data. The following questions were addressed in this chapter: 1) Are the groups supported by the two different analyses congruent? 2) Are there differences in the morphological and genetic level of variation within and between these groups?; and 3) How much morphological and genetic variation exists within a morphologically homogeneous population?

4.2 Materials and Methods

Plant material

The plant material considered for the comparison using morphological (Chapter 2) and AFLP analyses (Chapter 3) was restricted to single representatives of 23 different homogeneous populations from throughout the country which had been included in the

molecular analysis (Section 3.2.1, Table 3.1), plus 12 representatives of the morphologically homogeneous Cass population.

Assessment of morphological characters from the 23 different populations was mainly carried out using cultivated fresh material (cf), except for three OTUs, 159A/d, 148A/d, and 43A/d, which were examined in a dried state (d) from field collected material. The cultivation of these three specimens had not been successful with the plants dying before flowering. Flowering material was available from the field for OTU 148A/d and 43A/d, but not for 159A. The morphological characters were also examined on dried field material for representatives of the Cass population.

Similarity matrices

MVSP Plus Version 3.0 (Kovach 1998) was used for the analysis of molecular data. A total of 402 distinct AFLP fragments was revealed for the 23 OTUs representing 23 different populations. A presence-absence matrix for AFLP fragments was constructed, and a similarity matrix generated using the Jaccard's coefficient. A second Jaccard's similarity matrix was created for the 12 representatives of the Cass population based on a total of 227 AFLP fragments.

S-Plus 4.5 (r2) (Statistical Sciences 1998) was used for analyses of morphological data. Gower's similarity matrices were created by using the routine "Gower5" of the Phenetic Library for S-Plus 4.5 (r2) (Wilton 1999). A Gower similarity matrix (Gower 1971) was calculated for a morphological data set containing 23 OTUs and 80 characters (Table 2.5). The similarity matrix based on the 80 morphological characters for the 12 representatives of the Cass population was calculated also using Gower's general coefficient.

Correlation between the AFLP and morphological similarities

To test for significant correlation between the different data sets, and to compute the product-moment correlation, r, similarity matrices for morphological and molecular data were transferred to NTSYS-pc (Rohlf 1997) for analyses via the Mantel test (Mantel 1967).

UPGMA cluster analyses

Cluster analyses were carried out using the unweighted pair-group method of arithmetic averages (UPGMA) for both the morphological and molecular data sets, using the routine "phen.ana5" (Wilton 1999) in S-Plus 4.5 (r2). Additionally, this routine was used to calculate the cophenetic correlation coefficient for each phenogram. The cluster membership of OTUs and the similarities within and between clusters of the resulting phenograms were compared with descriptive methods.

4.3 Results

4.3.1 Representatives from different groups

Matrix correlation

The Mantel test showed a highly significant ($p < 0.001$) correlation between morphological and AFLP similarity matrices, with a matrix correlation statistic of $r = 0.589$.

UPGMA cluster analysis

The results of the cluster analyses of the morphological and AFLP data sets for the 23 representatives of different populations are shown in Figures 4.1 and 4.2. A comparison of the values for the overall cophenetic correlation coefficient for the two phenograms indicated that the phenogram based on AFLP data (0.91749 (Pearson) and 0.8675 (Spearman)) provided a much better representation of the original matrix than the phenogram based on morphological data (0.78941 (Pearson) and 0.75319 (Spearman)).

The cophenetic correlation plot for the phenogram based on AFLP data (Fig. 3.9) showed that the correlation between the similarity matrix and the phenogram remained relatively high (between 0.85 and 1.0) as OTUs were clustered, with no dramatic drop of the correlation coefficient occurring. Some linkages within the phenogram based on morphological data were in low correspondence with the similarity matrix as seen in the cophenetic correlation plot (Fig. 4.3).

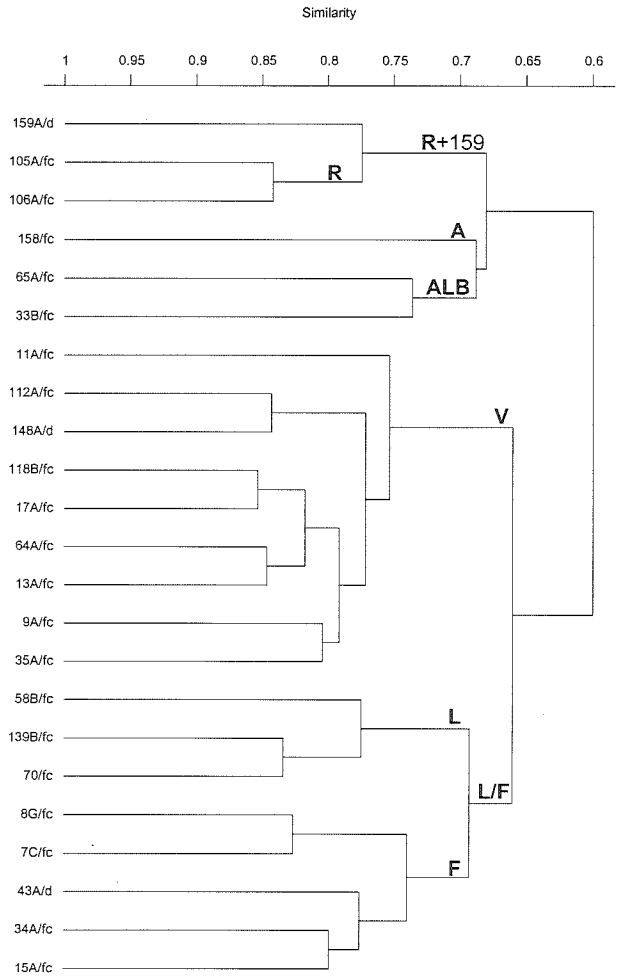


Fig. 4.1 UPGMA phenogram based on a reduced data set containing morphological data for the OTUs included in the molecular analysis.

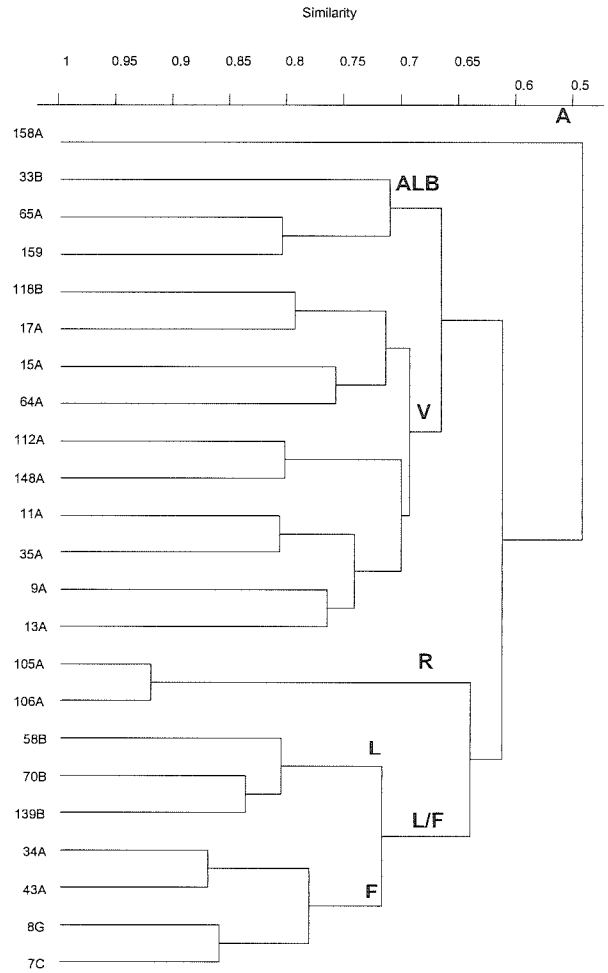


Fig. 4.2 UPGMA phenogram based on AFLP Data Set 2 (without *Cassinia aculeata*, *Ozothamnus rodwayi* and the Cass Population).

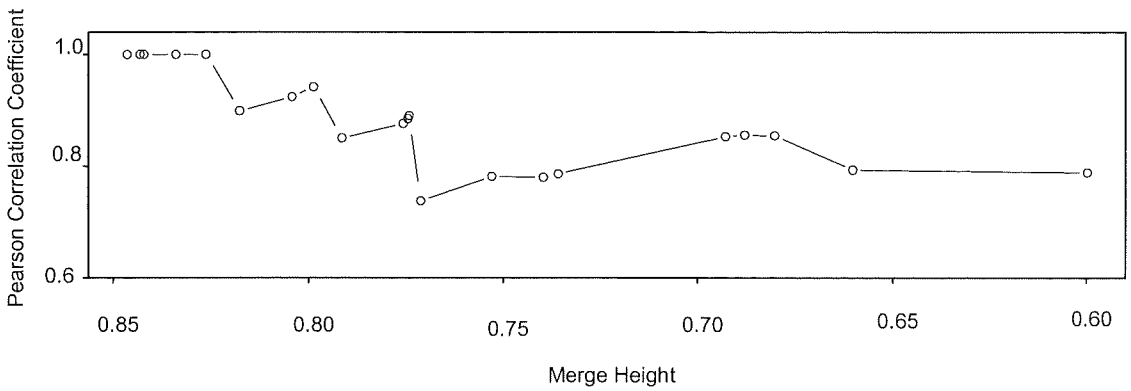


Fig. 4.3 Plot showing the change in the cophenetic correlation coefficient (Pearson) as OTUs are clustered in the UPGMA phenogram based on the reduced morphological data set containing 23 OTUs included in both the molecular and morphological study.

The overall structures of the two UPGMA phenograms generated from analyses of the morphological and AFLP data sets (Fig. 4.1 and Fig. 4.2) were remarkably similar. In both phenograms six clusters could be distinguished, although differences occurred in where and on which level of similarity these clusters were linked.

Cluster designations were as follows: A = 'Amoena' (representatives of the 'Amoena' group), ALB = 'Albida' (representatives of the 'Albida' group), V = 'Vauvilliersii' (representatives of the 'Vauvilliersii' group), R = 'Retorta' (representatives of the 'Retorta' group), and L/F = 'Leptophylla/Fulvida' with L= 'Leptophylla' and F= 'Fulvida' (representatives of the 'Leptophylla' group and the 'Fulvida' group).

Cluster membership of OTUs

In both analyses, the 'Leptophylla' and 'Amoena' clusters comprised identical OTUs. The composition of the 'Vauvilliersii' and the 'Fulvida' clusters were almost identical in both phenograms, with a few exceptions. OTU 15A/fc was assigned to the 'Fulvida' cluster based on morphology, and clustered within 'Vauvilliersii' based on AFLP data. A representative of the 'Albida' group (OTU 159A/d) was distantly linked to the 'Retorta' cluster, and the 'Albida' cluster contained only two OTUs (OTU 33B/fc and OTU 65A/fc)

in the phenogram based on morphology, while the representatives of the 'Retorta' group formed a clearly distinct cluster, and OTU 159 was assigned to the 'Albida' cluster in the phenogram based on AFLP data.

Similarities between and within groups

The phenogram based on AFLP data showed the representative of the 'Amoena' group (158A) as an outlier, joining the phenogram last at a low similarity level of 0.541, whereas morphological data linked OTU 158A/f at 0.688 to the 'Albida' group. The 'Amoena-Albida' cluster linked in the phenogram based on morphology to a cluster that comprised the representatives of the 'Retorta' group and OTU 159A/d, another representative of the 'Albida' group. The linkage of OTU 159A/d to the representatives of the 'Retorta' group, which formed a pair at a similarity of 0.842, was in low correspondence with the similarity matrix, indicated by a cophenetic correlation value of 0.74, the lowest value for any linkage within the phenogram based on morphology (Fig. 4.3).

The 'Amoena-Albida-Retorta' cluster (joined at 0.680) was linked to the rest of the phenogram based on morphological characters at 0.600, and no members of these groups showed affinities to the 'Vauvilliersii' or 'Leptophylla/Fulvida' clusters, as seen in the phenogram based on AFLP data. In the AFLP phenogram the 'Retorta' group linked at 0.639 with the 'Leptophylla/Fulvida' cluster, and the 'Albida' cluster joined to the 'Vauvilliersii' cluster at 0.665. The latter linkage did not correspond so well with the similarity matrix, as indicated by a cophenetic correlation value of 0.85; the lowest value for any linkages within the phenogram based on AFLP data (Fig. 3.9).

The 'Fulvida' and the 'Leptophylla' clusters were linked in both phenograms, but at slightly different levels of similarity. They were joined at 0.716 in the phenogram based on AFLP data and at 0.693 within the phenogram based on morphology. Within the 'Vauvilliersii' group the overall genetic distances were larger than those observed from morphology. The OTUs within the 'Vauvilliersii' cluster were linked on slightly lower levels of similarity based on AFLP data than on morphological data. The OTUs within this cluster formed similar subclusters in each phenogram. OTU 118B and OTU 17A, representatives of the 'Vauvilliersii S-Otago/Southland/Fiordland' group, were in both phenograms linked (at 0.853 based on morphology and at 0.792 based on AFLP data). The

two representatives of the 'Vauvilliersii North Island' group formed a pair in both phenograms (at 0.843 based on morphology and at 0.801 based on AFLP data). OTU 11A (originally assigned to the 'Vauvilliersii var. pallida/albida/canescens' group) was less genetically than morphologically distinct from the other representatives of the 'Vauvilliersii' group. It joined the 'Vauvilliersii' cluster as an outlier at 0.753 in the phenogram based on morphology, and was relatively closely linked (at 0.805) to OTU 35A ('Vauvilliersii Cant./Otago/N-Southland' group) in the phenogram based on AFLP data. The two representatives of the 'Vauvilliersii West Coast' group, OTU 9A and OTU 13A, formed a pair at 0.764 in the AFLP phenogram but not in the phenogram based on morphology, where OTU 13A and OTU 64A from Mt. Cook were linked at 0.846, and OTU 9A joined OTU 35A. The molecular data joined the specimen from Mt. Cook (OTU 64A) at 0.756 with one from the Canterbury Ranges (OTU 15A), which was assigned to the 'Fulvida' cluster in the phenogram based on morphology.

4.3.2 Variation within a homogeneous population

Matrix correlation

The results of the Mantel test showed that the matrix of genetic similarities within the Cass population was significantly correlated ($p < 0.001$) with the matrix based on the morphological characters for this population. The matrix correlation coefficient, $r = 0.405$, was slightly smaller than that for the matrices containing representatives of different populations.

UPGMA cluster analysis

Figures 4.4 and 4.5 are the UPGMA phenograms based on the morphological and AFLP data sets for the 12 representatives of the Cass population. The overall cophenetic correlation coefficient for the phenogram based on morphology was 0.71581 (Pearson) and 0.66748 (Spearman), for the phenogram based on AFLP data 0.84792 (Pearson) and 0.82744 (Spearman).

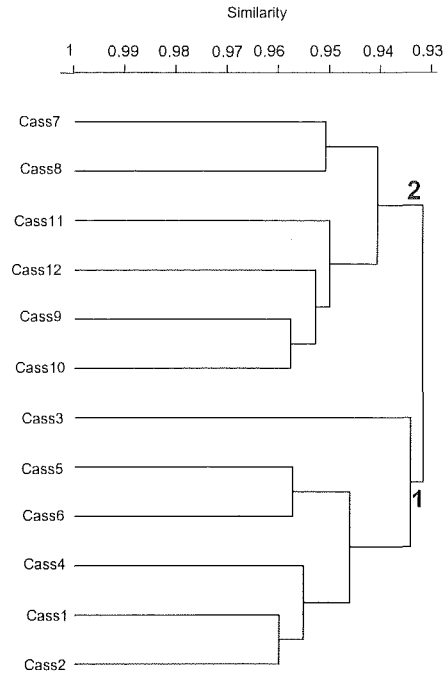


Fig. 4.4 UPGMA phenogram for the Cass population based on morphology.

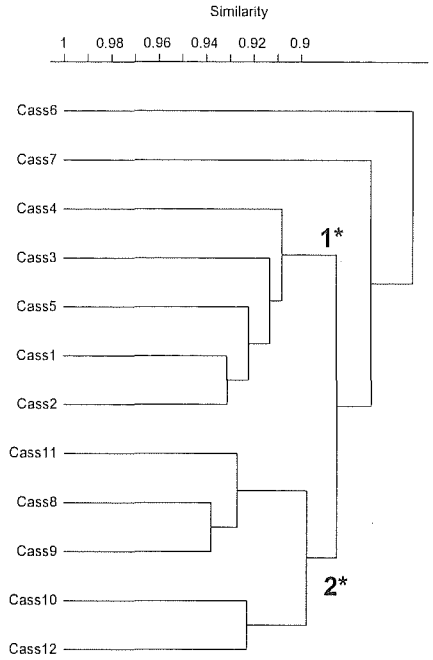


Fig. 4.5 UPGMA phenogram for the Cass population based on molecular data.

Plots showing the change in the cophenetic correlation coefficient (Pearson) as OTUs were clustered in the UPGMA phenograms are shown in Figures 4.6 and 4.7. Some linkages within both phenograms were in low correspondence with the similarity matrix as indicated by dramatic drops of the correlation coefficient.

The phenogram based on AFLP data (Fig. 4.5) consisted of two main clusters. One (Cluster 1*) contained the OTUs Cass2, Cass1, Cass5, Cass3, and Cass4; the other (Cluster 2*) comprised Cass12, Cass10, Cass9, Cass8, and Cass11. Both clusters were linked together at 0.885 and joined by two outlying OTUs, Cass7 at 0.870 and Cass6 at 0.853. The phenogram based on morphology (Fig. 4.4) was composed of two equally sized clusters (Cluster 1 and Cluster 2) joined at 0.932 and with almost identical OTUs as the two clusters in the phenogram based on AFLP data, with the exception that the two OTUs Cass6 and Cass7 outliers in the phenogram based on AFLP data were linked within Cluster 1 and Cluster 2 respectively.

There was a low agreement between the morphological and AFLP characters for the joining of OTUs within these main clusters. Only OTU Cass1 and OTU Cass2 formed a pair in both phenograms.

A small overall genetic and morphological divergence was indicated by similar high levels of similarity among OTUs (between 0.83 and 0.94 based on AFLP data, between 0.91 and 0.96 based on morphological data).

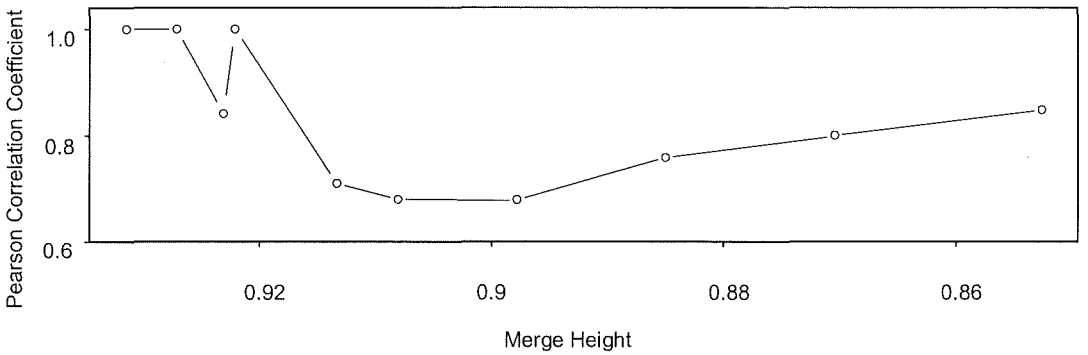


Fig. 4.6 Plot showing the change in the cophenetic correlation coefficient (Pearson) as OTUs are clustered in the UPGMA phenogram based on the molecular data for the Cass population.

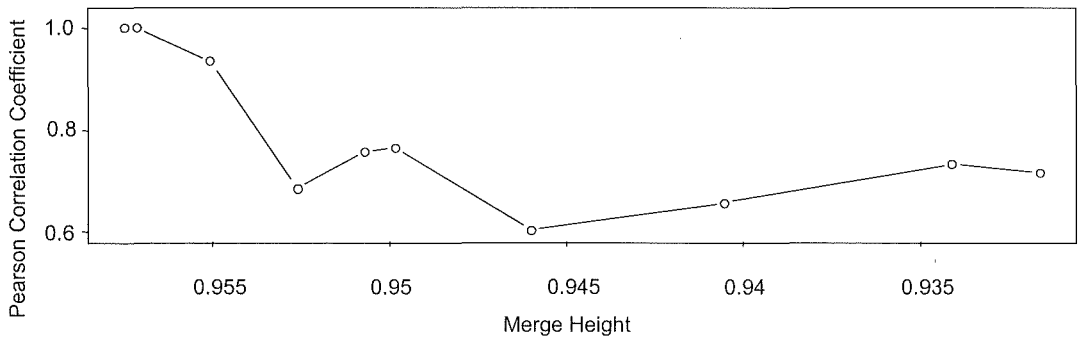


Fig. 4.7 Plot showing the change in the cophenetic correlation coefficient (Pearson) as OTUs are clustered in the UPGMA phenogram based on the morphological data for the Cass population.

4.4 Discussion

Given the likelihood of unequal rates of genetic and morphological divergence it is often recommended that both morphological and genetic data be considered in taxonomic treatments. The Mantel test used in the present study revealed significant correlations between genetic and morphological similarities for the data sets consisting only of individuals from the different populations. Although there are instances where data from different sources have resulted in no immediate resolution of taxonomic problems (e.g., Black-Samuelsson et al. 1997, Vijverberg 2001), studies often indicate congruence (e.g., Perrie et al. 2000, Van Huylenbroeck et al. 2000, Vaz Patto et al. 2001) or at least partial congruence between molecular and morphological data and therefore can greatly aid in taxonomic decision making. The correlation value $r = 0.589$ presented in the present study can be directly compared with the matrix correlation coefficient, $r = 0.47$ reported by Vaz Patto et al. (2001) for matrices based on morphology and AFLP data for *Hordeum chilense* species. However, this contrasts with Black-Samuelsson et al. (1997), who found little association between morphological and RAPD matrices for *Vicia pisiformis* when using the mantel test ($r = 0.129$). The correlation encountered in the present study, suggests that the different clusters represent distinct genetic groups of *Ozothamnus leptophyllus,* in that the genotypic differences between the groups are reflected both by morphological characters of the plants and AFLP fingerprinting patterns.

The association between the morphological and the AFLP data sets was also revealed by UPGMA (Fig. 4.1, Fig. 4.2) analysis. The clusters or groups revealed for *O. leptophyllus* by AFLP data were generally consistent with the results based on morphology. The major discrepancies between the two analyses were the placement of two OTUs, 159 and 15A, and the phenetic association between some of the groups. OTU 159, clearly associated with the 'Albida' group based on AFLP analysis, was distantly linked to the 'Retorta' group based on morphology. The linking of OTU 159 with the 'Retorta' group might have resulted from missing floral characters for this OTU, as explained in Section 2.4.5. OTU 15A from upper Cave Stream, Canterbury was originally assigned to the 'Vauvilliersii Cant./Otago/N-Southland' group (Section 2.3.1), and placed within the 'Vauvilliersii' cluster in those analyses of morphological characters which included high numbers of OTUs (Section 2.3.3.1). The reduction in the number of OTUs in numerical

analyses based on morphological characters resulted OTU 15A being placed in the 'Fulvida' cluster (Section 2.3.3.1, 2.3.3.2, 2.4.3). AFLP analysis supported the assignment of the OTUs regarded as having "unstable positions" (assigned to either the 'Fulvida' or the 'Vauvilliersii' cluster based on morphology, e.g. OTU 15A) to the 'Vauvilliersii' cluster. It is possible that these now homogeneous populations have resulted from ancient crosses between the two gene pools, but show stronger genetic affinities with the 'Vauvilliersii' group. This raises the question whether one should separate these populations from the 'Vauvilliersii' group.

AFLP analysis suggested that the 'Retorta' group is genetically similar to the 'Leptophylla/Fulvida' group. This was not shown in the analysis of the morphological characters, at least not when both floral and vegetative characters were included. However, when only vegetative characters were analysed, the 'Retorta' group clustered with the 'Leptophylla' group, which may have been due to overlapping ranges of some of the vegetative characters (Section 2.3.3.4).

The morphological similarity between the 'Amoena' and 'Albida' groups was not reflected in the AFLP data, which placed the 'Amoena' group in an outlier position. Considering that the 'Amoena' group is known only from cliffs from Kerr Point to North Cape, whilst the 'Albida' group can be found only in montane and subalpine shrubland and grassland in the Kaikoura Ranges of the South Island, it is not surprising to find evidence in support of homoplasy of morphological characters rather than close genetic relationships.

The representatives of the 'Albida' group formed a distinct cluster, in both morphological and molecular analyses. However, within the group they were only distantly linked with each other. This indicated that there is genetic and morphological variation in this entity. The association of the 'Albida' group with the 'Vauvilliersii' group revealed by analysis of the AFLP data was at least partially supported by morphological analyses. Analyses of those morphological data sets with more OTUs (Section 2.3.3.1, 2.3.3.2), including intermediate forms, led to groupings similar to those observed in the analysis of the molecular data. Morphological analysis also revealed the presence of intermediate forms, probably of hybrid origin, between these two groups (Section 2.3.3.1).

In both morphological and molecular analyses, representatives of the 'Vauvilliersii' group linked quite distantly with each other on comparatively low levels of similarities.

The genetic distances between representatives of different populations within this cluster were even higher than distances based on morphology, indicating that the 'Vauvilliersii' group is highly variable. This is not surprising considering the wide geographic distribution of this group. The formation of subgroups may be warranted if the results of the morphological analysis are to be considered. Although molecular analysis could not confirm the distinctiveness of these subgroups, perhaps because they were not well represented in the AFLP study, they might still be considered as distinct taxa within a 'Vauvilliersii' taxon, based on morphology and distribution (refer to Chapter 5).

The 'Fulvida' and the 'Leptophylla' groups were joined at a high level of similarity relative to that found between any of the other groups, based on AFLP data. They were, therefore, not recognised as distinct groups in the analysis of molecular data (Section 3.3.3). However, morphological data were able to distinguish between these two groups. Moreover, although there was a high genetic similarity between the two groups, there was very little genetic variation within each group, i.e. the members of each group were linked at high levels of similarity (between 0.874 and 0.780). This left a comparatively large phenetic gap between the linkages within each cluster, and the joining of the 'Fulvida' and the 'Leptophylla' cluster at 0.716.

Another objective of this part of the study was to compare the morphological and genetic variation within a morphologically homogeneous population. Both morphological and genetic variation patterns were studied within the Cass population. The median Gower similarity for the morphological data set of 12 individuals of this population was 0.93. The result of the AFLP data analysis showed also little resolution among individuals of this homogeneous population, with a median Jaccard similarity of 0.89. This can be interpreted as the occurrence of ongoing genetic exchange within this population.

UPGMA analysis and matrix comparison suggested a slightly lower congruence between genetic and morphological variation among the individuals of the Cass population, compared to that between genetic and morphological variation from different populations. The Mantel test results demonstrated that the genetic similarity (based on AFLP data) and the morphological similarity (based on morphological characters) are significantly ($p < 0.001$) correlated. The correlation coefficient for the morphological and AFLP similarity matrices was lower for data sets derived from the Cass population ($r = 0.405$) than for those derived from representatives of different populations ($r = 0.589$). However, even

within a population, molecular and morphological analysis showed rather high similarities, and the morphological variation seems to be, at least to a certain extent, congruent with the genetic variation.

The relatively low agreement between morphology and AFLP characters joining OTUs of the Cass population within the two main clusters in UPGMA analysis might be, at least partially, due to difficulties in transforming similarity matrices with little variation into a phenogram. This is also indicated by low overall cophenetic correlation coefficients, reflecting a poor fit of the phenograms to the data. Another possible explanation is that only a few genes may underlie the individual characteristics (Gottlieb 1984; Kadereit 1994) and that these are not sufficiently detected by the AFLP markers, which are considered as neutral and located in non-coding DNA.

In conclusion, there is good congruence between the results of molecular and morphological analyses of *O. leptophyllus* in New Zealand indicating there is a genetic basis for associations inferred from analyses of these data. Clusters of taxa resulting from these analyses will be considered for taxonomic revision.

Chapter 5

Revision of the *Ozothamnus* (Compositae) complex in New Zealand

The aim of the study was to resolve the taxonomic problems present in the *Ozothamnus leptophyllus* complex and to provide a new classification for this complex by determining the most appropriate taxonomic status for any recognisable entities. It is not a simple task to generate a satisfactory classification for *Ozothamnus leptophyllus* in New Zealand. Recognition of taxa is hampered by continuous variation of most quantitative morphological characters and a paucity of constant qualitative features, a fact that becomes quite obvious with study of the overall diversity present within the complex. Nevertheless, a certain level of morphological and genetic distinction could be detected and some morphological differences were found. This led to the recognition of six groups.

Circumscription of groups

'Amoena' group

Analysis of the AFLP data reveals that the single included representative of the 'Amoena' group is genetically distinct from the other specimens of *Ozothamnus* included in this study. Jaccard similarity values between the representative of the 'Amoena' group and any other specimens are lower than 0.59 (Section 3.3.3).

The overall morphological distinctness of the 'Amoena' group is due to a combination of characters (Section 2.4.8) such as the small overall size, the absence of receptacle scales, large (6-13(-18) × 2.5-4.5 mm) narrow-obovate leaves, an extremely dense tomentum on the abaxial surface of the leaves, and densely arranged twin hairs and glandular hairs on the achenes. However none of these character states is unique to the 'Amoena' group. The 'Vauvilliersii' group also contains some very small specimens. Leaf shapes and sizes similar to that found in the 'Amoena' group are present in the

'Vauvilliersii' and 'Albida' groups. Tomentum of similar density to that of the 'Amoena' group can be found in the 'Albida' group. Some representatives of the 'Fulvida' group lack receptacle scales as in the 'Amoena' group.

The representatives of the 'Amoena' group and those of the 'Albida' group (corresponding with *C. vauvilliersii* var. *albida* Kirk) have many vegetative character states in common. The similarity of the 'Amoena' group to the 'Albida' group indicated by the analysis of morphological characters is interesting, considering that the former is only known from cliffs from Kerr Point to North Cape in the extreme north of the North Island, and the latter is restricted to montane and subalpine shrubland and grassland in the Kaikoura Ranges of the South Island. Therefore, the character similarities between them have either been maintained, or have developed independently. Cheeseman (1906) remarked that *Cassinia amoena* (which corresponds with the 'Amoena' group discussed here) has much of the aspect of *C. vauvilliersii* var. *albida* from which it is distinguished by the smaller size, narrower heads, fewer florets, and absence of the receptacle scales.

Vegetative and floral characters, such as different habit and much smaller size, larger leaves and the absence of receptacle scales, are found to separate the 'Amoena' group from the 'Leptophylla' and 'Retorta' groups. Cheeseman (1906) also found these characters useful in distinguishing *Cassinia retorta* and *C. leptophylla* from *C. amoena*. Kirk (1899) and Cheeseman (1906, 1925) used the absence of receptacle scales as a key character to distinguish *C. amoena* and *C. fulvida* from *C. retorta*, *C. leptophylla*, and *C. vauvilliersii*, and separated *C. amoena* and *C. fulvida* using leaf size, leaf shape, and tomentum. Allan (1961) employed a combination of plant size, leaf size, leaf tomentum, capitulum shape, floret number per capitulum, number of receptacle scales, achene tomentum, and involucre tomentum as key characters to separate *C. amoena* from the other *Cassinia* species.

'Retorta' group

The results of the AFLP analysis show that the 'Retorta' group is a distinct entity. The two representatives included in molecular studies cluster together with a Jaccard similarity of 0.92, whilst the mean similarity to the other *Ozothamnus* specimens is 0.62. AFLP analysis also indicates affinities of the 'Retorta' group to the 'Leptophylla/Fulvida' group (Section

3.3.3). The representatives of the 'Retorta' group are shown to have a unique set of morphological characters leading to their positioning in cluster analyses without an obvious affinity to any of the other groups (Section 2.3.3). The arrangement of only a few (6-11) large capitula in small, almost simple corymbs is a unique character for the 'Retorta' group. High floret numbers per capitulum (12-18 on average, and in some capitula even more) are also found to be characteristic for the 'Retorta' group but similar numbers could be found for the 'Albida' group, which is in nearly all other characters quite different to the 'Retorta' group. Floral characters, such as larger floral parts in relatively high numbers, distinguish the 'Retorta' group clearly from the 'Leptophylla' and the 'Fulvida' groups with which it shares most vegetative characters (see below).

Although the representatives of the 'Retorta' group are shown to form a distinct group, the similarities of this group to others are difficult to determine based on cluster analyses of morphology. Analyses of morphological characters in this study show that the ranges of some vegetative characters such as leaf size, leaf apex, leaf tomentum and leaf spacing overlap to a certain extent. This leads to a close linkage of the 'Retorta' group with the 'Leptophylla' group in the numerical analysis based on vegetative characters, but the positioning in cluster analysis based on a complete set of morphological characters indicates the overall dissimilarity of the 'Retorta' group to any other group.

Like the 'Amoena' group, the 'Retorta' group corresponds directly with a previously described *Cassinia* species, *C. retorta* A. Cunn. ex DC. According to Cunningham's (1837) description, *C. retorta* is similar to *C. leptophylla*. Hooker (1852) also noted the resemblance of *C. retorta* to *C. leptophylla*, but remarked on some differences in *C. retorta* such as the more robust habit, leaves which are broader, more obovate and more constantly recurved, panicles of only a few (3-8) heads with very woolly pedicels, and the larger capitula. In this study high floret numbers per capitulum (12-18 on average, and sometimes more) are found to be characteristic for representatives of the 'Retorta' group. Review of the literature (Table 2.1) found conflicting reports of floret number per capitulum for *C. retorta*, including 15-16 (Cunningham 1837), 8-10 (Hooker 1852), ~8 (Kirk 1899), 6-20 (Cheeseman 1906, 1925), and 10-20 (Allan 1961). However, all authors agreed that the capitula of *C. retorta* are arranged in small panicles. The number of capitula per panicle has been reported as 1-8 (Hooker 1864) and 3-8 (Kirk 1899).

Hooker (1867) used the capitulum number as key character. Similar capitulum numbers per panicle (6-11) are found for the representatives of the 'Retorta' group in this study.

The tomentum of *Cassinia retorta* is said to be white or whitish (Hooker 1864; Kirk 1899; Cheeseman 1906, 1925) and not overlain with yellow exudates. Allan (1961) added to the description of the leaf tomentum of *C. retorta* "sometimes yellowish". Similar observations were made by Carse (1930) and Kalin (1967b). However, these authors pointed out that the colour of the tomentum on branchlets and leaves of *C. retorta* is variable according to geographic area, i.e. it is much more yellow in the extreme north of Auckland Province than further south where it is usually whitish. During this study several specimens with yellow coloured exudates were encountered. The tomentum colour or the absence of yellow exudates is therefore of dubious quality as a key character, despite having been used by Hooker (1864), Kirk (1899), and Cheeseman (1906, 1925). Allan (1961) used leaf shape and size, recurved margin and pappus hairs to distinguish *C. retorta* from the other *Cassinia* species. These characters do not separate the 'Retorta' group from the 'Leptophylla' group in this study.

'Albida' group

Analysis of the AFLP data supports the 'Albida' group (Section 3.3.3). The members cluster together with a Jaccard similarity of 0.74. The mean similarity of the 'Albida' group to the other *Ozothamnus* specimens in the molecular study is 0.633.

Large (6-13 × 2.5-4 mm), obovate, acute leaves with a very dense white tomentum which is not or very sparsely overlain with yellow exudates on the abaxial surface, combined with large capitula with many (c. 17) florets arranged in large, slightly spreading panicles distinguish the 'Albida' group from the other groups.

The similarity of the 'Albida' group to the 'Vauvilliersii' group indicated by analysis of molecular data (Section 3.3.3) is at least partially supported by morphological analyses (Section 2.3.3). The 'Albida' group shows some similarities to the 'Amoena' group and to the 'Vauvilliersii' subgroup which includes the specimens formerly described as *C. vauvilliersii* var. *pallida* Allan. The ranges of the vegetative characters of the representatives of the 'Albida' group overlap to a limited extent with those of the representatives of the 'Amoena' and 'Vauvilliersii' groups. The members of the 'Albida' group are distinguished from those of the 'Fulvida' group by larger leaves and a higher

number of receptacle scales per capitulum. The ranges for the colour and tomentum density found in this study for the 'Albida', 'Leptophylla' and 'Amoena' groups are very similar, but they separate the 'Albida' group from the 'Vauvilliersii' group. This is partially supported by the work of Cockayne (1906), who raised Kirk's (1899) *Cassinia vauvilliersii* var. *albida* to species level, based on the distinct tomentum of the under surface of the leaf, which is white or yellowish-white, and not fulvous as in *C. vauvilliersii*. Cockayne (1906) also mentioned that *C. albida* has larger leaves than *C. vauvilliersii*, which is in accord with the results presented in this study (Section 2.3.3.2). Other characters differentiating the 'Albida' group from the 'Vauvilliersii' group are thick pappus hairs that do not spread much at the tips, and short basal appendages of the anthers.

Cockayne (1906) described *Cassinia albida* var. *canescens*, which is distinguished from *C. albida* var. *albida* by a more dense and obvious tomentum on the adaxial surface of the leaves. However, Allan (1961) did not accept Cockayne's *C. albida* as a species and *C. albida* var. *canescens* appears in his flora as *C. vauvilliersii* var. *canescens* (Section 1.1). From the material examined in this study it seems very unlikely that two distinct entities exist; single plants are found in which separate branches were representative of each of the varieties '*albida*' and '*canescens*'. None of the plants in this group loses the tomentum on the adaxial surface of the leaves. This character that was used by Allan (1961) to distinguish between the two varieties. An increased amount of leaf surface waxes might make the presence of the hairs less obvious in older leaves.

'Vauvilliersii' group

This group is formed by specimens previously identified as *Cassinia vauvilliersii* (Hombr. et Jacq.) Hook.f., *C. vauvilliersii* var. *rubra* (Buchanan) Kirk or *C. rubra* Buchanan, *C. vauvilliersii* var. *pallida* Allan, *C. fulvida* var. *montana* Allan, and *C. fulvida* Hook.f. pro parte.

Molecular and morphological data support the recognition of the 'Vauvilliersii' group. The level of genetic diversity is quite high within the 'Vauvilliersii' group, the mean Jaccard similarity within this group being 0.692. Molecular data link the 'Vauvilliersii' group most closely to the 'Albida' group (Section 3.3.3).

Analyses of morphological data indicate a similarity between the 'Vauvilliersii' group and the complex consisting of the 'Fulvida' group and the 'Leptophylla' group

(Section 2.3.3). The 'Vauvilliersii' group may be morphologically distinguished from the 'Fulvida' group by broader leaves, larger capitula and floral parts and numerous receptacle scales among the florets. From the 'Retorta' group it is distinguished by having larger leaves, often several compound panicles with smaller capitula, and fewer florets per capitulum. Larger size, the presence of dense yellow exudates on branchlets and leaves, more florets per capitulum and the presence of numerous receptacle scales among the florets readily distinguish the representatives of the 'Vauvilliersii' group from that of the 'Amoena' group. The 'Vauvilliersii' group can be distinguished from the 'Albida' group by a higher density of yellow exudates and a less dense tomentum on the abaxial surface of the leaves. The representatives of the 'Vauvilliersii' group also have slightly smaller leaves, more slender pappus hairs that are spreading at the tips, and longer anther tails compared to the 'Albida' group.

Analyses of morphological data identify two main subgroups within the 'Vauvilliersii' group. The 'Vauvilliersii var. pallida' subgroup comprises the specimens corresponding to the previously described *C. vauvilliersii* var. *pallida*. This subgroup is distinguished mainly by a denser tomentum on leaves and branchlets, which is overlain by a smaller amount of less brightly coloured pale yellow-green exudates. Smaller capitula and smaller, sometimes obtuse or rounded leaves distinguish the 'Vauvilliersii var. pallida' subgroup from the 'Albida' group. Representatives of the 'Vauvilliersii var. pallida' subgroup have a less dense tomentum on the abaxial surface of the leaves than those of the 'Albida' group. The similarity between the 'Vauvilliersii var. pallida' subgroup and the 'Albida' group becomes obvious during numerical analysis of the morphological data (Section 2.3.3.1) and intermediate forms between these groups could be discerned. This is supported by the fact that hybrids have been reported (Cockayne and Allan 1934). Allan (1961) noted that "within the *Vauvilliersii* complex the vars *pallida* and *albida* often meet and produce polymorphic progeny".

Within the second subgroup of the 'Vauvilliersii' group, the North Island representatives are slightly separated from the South Island representatives and the Fiordland/Southland representatives form a small subunit (Section 2.3.3). Plants from Fiordland and Southland, as well as some specimens from slightly west of the Main Divide and from the North Island, were found to correspond to Buchanan's (1887) description of *Cassinia rubra*, with its characteristic red-tinged outer involucral bracts. Cheeseman

(1906) reduced *C. rubra* to *C. vauvilliersii var. rubra* and Allan (1961) remarked that involucral bracts tinged with red in the apical parts are not infrequent in both *C. vauvilliersii* and *C. fulvida*, an observation which was confirmed during this study. This character is not consistent within an otherwise homogenous population at one time and also tends to vary seasonally.

Analyses of morphological and molecular data clearly assign specimens from montane shrubland and herb-moore communities from high altitudes in Canterbury (Mt. Peel: OTU 35A), Westland (Otira Valley: OTU 13A and OTU 13B) and North-West Nelson (Garibaldi Ridge: OTU 9A) to the 'Vauvilliersii' group (Sections 2.3.3 and 3.3.3). They are placed within the second subgroup of the 'Vauvilliersii' group based on morphology (Section 2.3.3). These specimens were previously identified as *Cassinia fulvida* var. *montana* Allan. Kalin (1967b) remarked that it seems more probable that, based on morphological characters and ecological affinities, *C. fulvida* var. *montana* is simply a Canterbury form of *C. vauvilliersii*.

Other OTUs from mountain areas in the South Island such as OTU 15A (from upper Cave Stream, Canterbury), and OTUs originated from Cass, Canterbury (e.g. OTU 4X), were originally assigned to the 'Vauvilliersii Cant./Otago/N-Southland' group (Section 2.3.1), and placed within the 'Vauvilliersii' cluster in analyses of morphological characters which included larger numbers of OTUs (analyses of Data Sets 1-3, Section 2.3.3.1). The reduction of the number of OTUs in numerical analysis based on morphological characters caused OTU 15A and OTU 4X to be placed in the 'Fulvida' cluster (analysis of Data Set 4, Section 2.3.3.2, 2.4.3). These OTUs are suspected to have an intermediate status between the 'Vauvilliersii' group and the 'Fulvida' group. Principal coordinate analyses supports this theory (Section 2.3.3.2, 2.4.4). Microcharacters (Section 2.3.3.3) and vegetative characters (2.3.3.4) direct the assignment of OTU 15A and OTU 4X to the 'Vauvlilliersii' group, but a separate analysis of the floral characters groups them together with the representatives of the 'Fulvida' group. (Section 2.3.3.4, 2.4.7). Only OTU 15A represented this subgroup of the 'Vauvilliersii' group in the molecular analysis and AFLP analysis supports the assignment of this OTU to the 'Vauvilliersii' group. The similarity values between OTU 15A and the representatives of the 'Fulvida' group ranged from 0.61 to 0.65. Similarity values up to 0.76 between OTU 15A and representatives of the 'Vauvilliersii' group supported the assignment of this OTU to the 'Vauvilliersii' group.

These populations from the montane areas of the South Island, morphologically distinguished from the remainder of the 'Vauvilliersii' group by narrower leaves and slightly smaller capitula can be circumscribed as the "narrow-leaved subgroup of the 'Vauvilliersii' group", and may be seen as a subtaxon within a 'Vauvilliersii' taxon. They are distinguished from the representatives of the 'Fulvida' group by a more compact habit, smaller panicles with larger capitula and more receptacle scales among the florets. The new branchlets develop below the old terminal inflorescence after fruiting and not while the flowers are still in bud as in representatives of the 'Fulvida' group.

'Fulvida' group

Morphological and molecular data support the recognition of the 'Fulvida' group as distinct (Sections 2.3.3 and 3.3.3) even if it is similar overall to the 'Leptophylla' group (previously described as *Cassinia leptophylla* (G.Forst.) R.Br. Analysis of AFLP data results in high similarity values (mean 0.807) among the representatives of the 'Fulvida' group. Although AFLP data link the 'Fulvida' group with the 'Leptophylla' group at a similarity level of 0.750 a large gap is present between the 'Fulvida' group and the tightly clustered 'Leptophylla' group (Section 3.3.3).

The overall morphological distinctiveness of representatives of the 'Fulvida' from the 'Leptophylla' group can be attributed to striking differences in the colour of their leaf and branchlet tomentum due to differences in tomentum density and thickness and exudate density and colour. Representatives of the 'Fulvida' group have a less dense tomentum that is overlain by yellow exudates. Vegetative characters other than colour did not separate the 'Fulvida' and the 'Leptophylla' groups. Kalin (1967a) mentioned that the average leaf length and width overlap completely in pure populations of *C. fulvida* and *C. leptophylla* examined in her study.

Most of the floral characters have similar or overlapping ranges for the 'Fulvida' and 'Leptophylla' groups. However, the representatives of the 'Fulvida' group have fewer florets and receptacle scales per capitulum compared with those of the 'Leptophylla' group. These characters were also used by Kirk (1899) and Cheeseman (1906, 1925) to distinguish *Cassinia fulvida* from *C. leptophylla* and *C. retorta.* Allan (1961) reported similar floret numbers for *C. fulvida* and *C. leptophylla.*

The morphological similarity of these two groups found in this study is supported by Hooker (1852), who recognised *C. fulvida* as *C. leptophylla* var. γ (Section 1.1). Hooker (1852) described it as a plant very similar to *C. leptophylla* but with the yellow and glutinous foliage and young leaves of *C. vauvilliersii*. Hooker (1864) promoted var. γ to species level, but remarked that he was still doubtful as to the validity of this species.

The 'Fulvida' group does not correspond entirely with *Cassinia fulvida* Hook.f. since it does not include the entity previously described as *C. fulvida* var. *montana* Allan, nor the larger leaved representatives of *C. fulvida* from montane shrubland. These were assigned to the 'Vauvilliersii' group (see above).

The 'Fulvida' group is morphologically distinguished from the 'Amoena', 'Albida', 'Vauvilliersii' and the 'Retorta' groups by small capitula with only a few (5-9) small florets, arranged in large spreading panicles, a more upright slender growth form with long shoots, greater plant height, a smaller branchlet angle, and a denser branchlet spacing.

'Leptophylla' group

Like the 'Fulvida' group the 'Leptophylla' group is found to represent a distinct entity mainly based on morphological data, but also supported by molecular data which cluster the representatives of the 'Leptophylla' group on a high level of similarity (mean 0.817). It is morphologically distinguished from the 'Fulvida' group by a denser tomentum that is not or far less, overlain with yellow exudates. Representatives of the 'Leptophylla' group have a wider range of floral counts with a tendency for more florets per capitulum and more receptacle scales per capitulum compared to the representatives of the 'Fulvida' group.

Members of the 'Leptophylla' group are not only closely allied to the 'Fulvida' group as described above, but also showed some similarities to the 'Retorta' group in respect of vegetative characters, as seen in the results of numerical analysis based on vegetative characters (Section 2.3.3.3).

Distribution and habitats

Ozothamnus leptophyllus is distributed throughout New Zealand's main islands from North Cape to Bluff and also occurs on Stewart Island and the Auckland Islands. It is found in many ecological situations (Section 1.3.2), and Cockayne (1928) and Wardle (1991) describe a wide range of different vegetation types in which *O. leptophyllus* is present. Both authors recognised different "species" (as *Cassinia* species, Cockayne 1928) or different "forms" (Wardle 1991). The groups recognised in this study correspond largely with previously recognised taxa, and their distribution and habitat will be summarised only briefly.

The representatives of all groups have a high light intensity requirement. They are therefore present only in areas of open scrub or thicket, never in dense closed scrub or forest. Each group has "original" habitats without permanent human habitation or modification, in which it occurs as an early shrub species, or sometimes as a pioneer, of a primary succession. The vast majority of plant communities with *Ozothamnus leptophyllus* represent stages of secondary succession or, according to Cockayne (1928) they are "indigenous-induced".

'Amoena' group (Fig. 5.1 A)

The 'Amoena' group is the northernmost group, known only from the North Cape area between Surville Cliffs and Kerr Point. On coastal ultramafic rock outcrops it is one of the early shrubs in primary succession and a component of natural plant communities in this habitat. In the vicinity of the coastal cliffs, it can be found on clay soils derived from ultramafic rocks within low, fire-modified scrub, dominated mainly by *Leptosperum scoparium* (Cockayne 1928, Wardle 1991).

'Retorta' group (Fig. 5.1 B)

The 'Retorta' group occupies the Auckland, Taranaki and Gisborne provincial areas as far south as latitude 40°30'S on the west coast and 38°S on the east coast. It occurs in (unmodified) coastal habitats such as shrub dunes, fixed dunes, hollows and sand-plains. Representatives of the 'Retorta' group also invade grasslands in the vicinity of coastal habitats, being more tolerant of coastal exposures and frequent burning than *Leptospermum scoparium* and *Kunzea ericoides*. It is in these "indigenous-induced" plant communities in

the North Cape region in the vicinity of coastal cliffs between Surville Cliffs and Kerr Point where hybrids are found between the 'Retorta' and 'Amoena' groups. At approximately latitude 37°30'S, the 'Retorta' group overlaps in its distribution with the 'Leptophylla' group.

'Leptophylla' group (Fig. 5.1 C)

The 'Leptophylla' group is distributed in the central portion of New Zealand, occurring in Taranaki, Gisborne, Hawkes Bay, the Cook Strait area, northern Marlborough, and in parts of Nelson. From latitude 37°30'S, it extends southwards coastally to latitude 40°30'S on the west coast of the South Island and to latitude 42°S on the east coast of the South Island. The 'Leptophylla' group is the ecological equivalent of the 'Retorta' group. It is predominantly coastal but extends inland at the Kaikoura Ranges as a member of "indigenous-induced" plant communities.

'Fulvida' group Fig. (5.1 D)

The 'Fulvida' group is restricted to the South Island, from latitude 41°30'S southwards to latitude 46°S. It occurs in coastal and lowland habitats, and extends inland where it occurs along riverbeds and in "indigenous-induced" shrubland and *Leptospermum-Pteridium* (manuka-bracken) communities of the lowlands and lower hill vegetation (lowland-montane belt). Communities with representatives of the 'Fulvida' group are often present in areas of maximum dryness and are frequently dominant on poor soils. This is likely to be more a consequence of the absence of competitors than a preference for less fertile soils.

'Vauvilliersii' group (5.1 E)

The 'Vauvilliersii' group has the widest latitudinal and altitudinal range of any of the groups. It occurs on islands in the Hauraki Gulf, extends from 37°S southwards on the main islands and can be also found on the Auckland Islands. It ranges from montane and subalpine regions in the North Island and the Southern Alps down to sea level in South Otago, Southland, Fiordland, Stewart Island, and on the Auckland Islands. It is quite common immediately east of the Main Divide on the South Island, where it occurs in *Hebe* scrub and shrub-composite scrub that requires a high rainfall for its full development. Only a few scattered populations can be found in the mountain ranges of the West Coast of the South Island. The 'Vauvilliersii' group occurs as an early species of a primary succession

at edges of riverbeds, slips and avalanche tracks, and can be found in high mountain communities such as scrub communities, mixed communities (shrubs, herbs, semi woody plants, grasses etc.) and herb-moor communities. Dominance of representatives of the 'Vauvilliersii' group usually indicates the area has had a history of burning, which encourages the growth of low shrubs, tussock grasses and large herbs. Representatives of the 'Vauvilliersii' group are a common, sometimes dominant component of subalpine secondary heath as a form of high-altitude secondary succession. On southern borders of the Otago plateau representatives of this group occur in mountain wetlands and cushion bogs, and even further south it is a part of shrubland communities of the lowlands and lower hill vegetation (lowland-montane belt) which occur under diverse conditions.

Narrow-leaved subgroup of the 'Vauvilliersii' group (Fig. 5.1 F)

In montane shrubland and grassland east of the Main Divide from latitude 42°30'S southwards on the mountain ranges of Canterbury, Otago and Southland, the typical representatives of the 'Vauvilliersii' group are replaced by the narrow-leaved subgroup which descends to the lowlands in the southern part of its range.

'Vauvilliersii var. pallida' subgroup (Fig. 5.1 G)

Between 41°S and 42°30'S, the above narrow-leaved subgroup of the 'Vauvilliersii' group is replaced by the 'Vauvilliersii var. pallida' subgroup. Both of these subgroups form more or less regional populations within the range of the 'Vauvilliersii' group and occur in similar habitats.

'Albida' group (Fig. 5.1 H)

The 'Albida' group is confined to the Kaikoura Mountains and their vicinity, where it occurs along the seaward face, extending in a westerly direction as far as the Clarence River and the middle portion of the Wairau Valley. It is a component of subalpine scrub communities. Throughout its subalpine distribution, hybrids with the 'Vauvilliersii' groups occur, due to the invasion of representatives of the 'Vauvilliersii' groups into the range of the 'Albida' group. Representatives of the 'Albida' group are not only found in the subalpine scrub, but descend to the river-flats on the Kaikoura Plains where the 'Albida' group is known to hybridise with the 'Leptophylla' and 'Fulvida' groups.

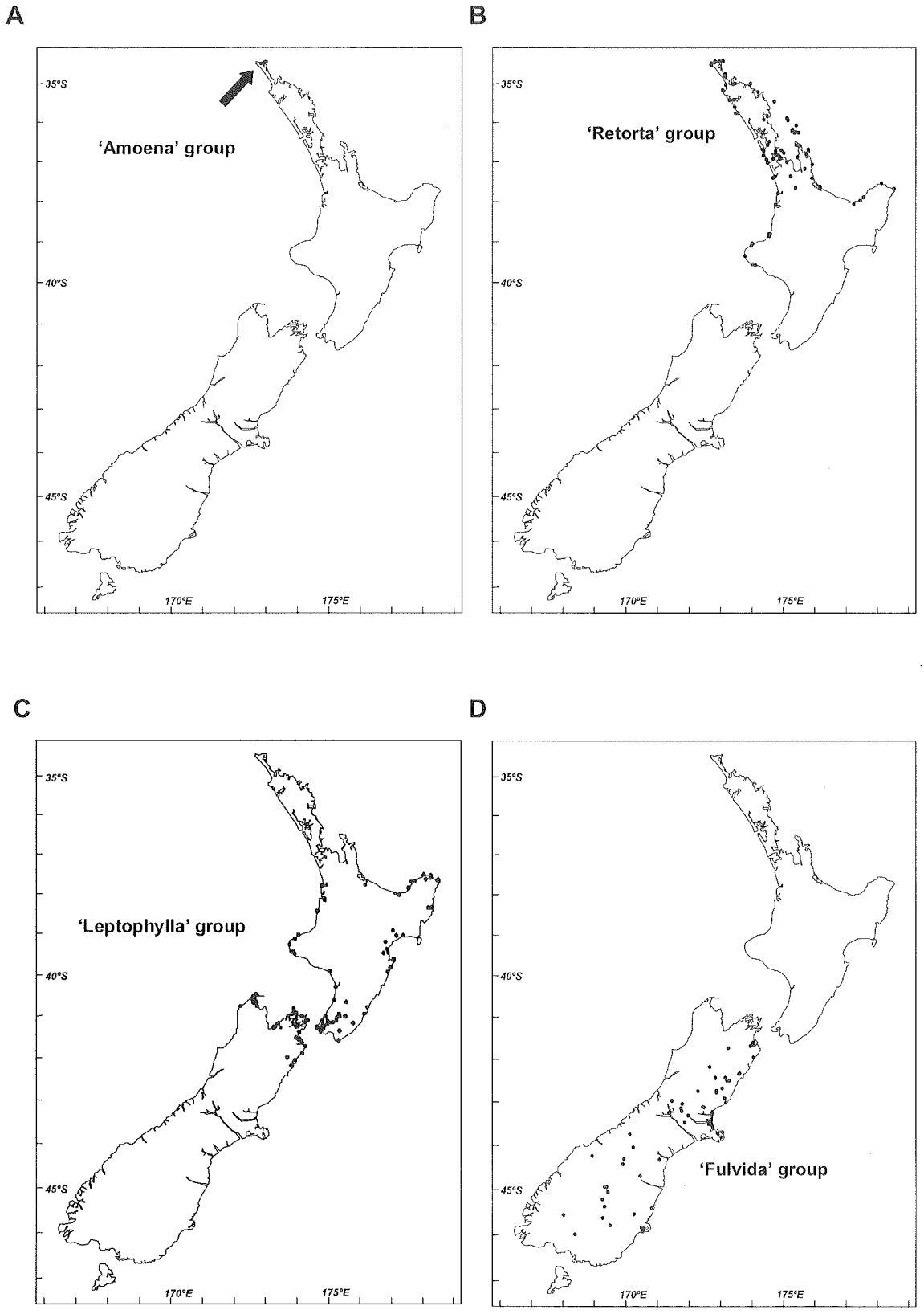


Fig. 5.1 see caption on page 230.

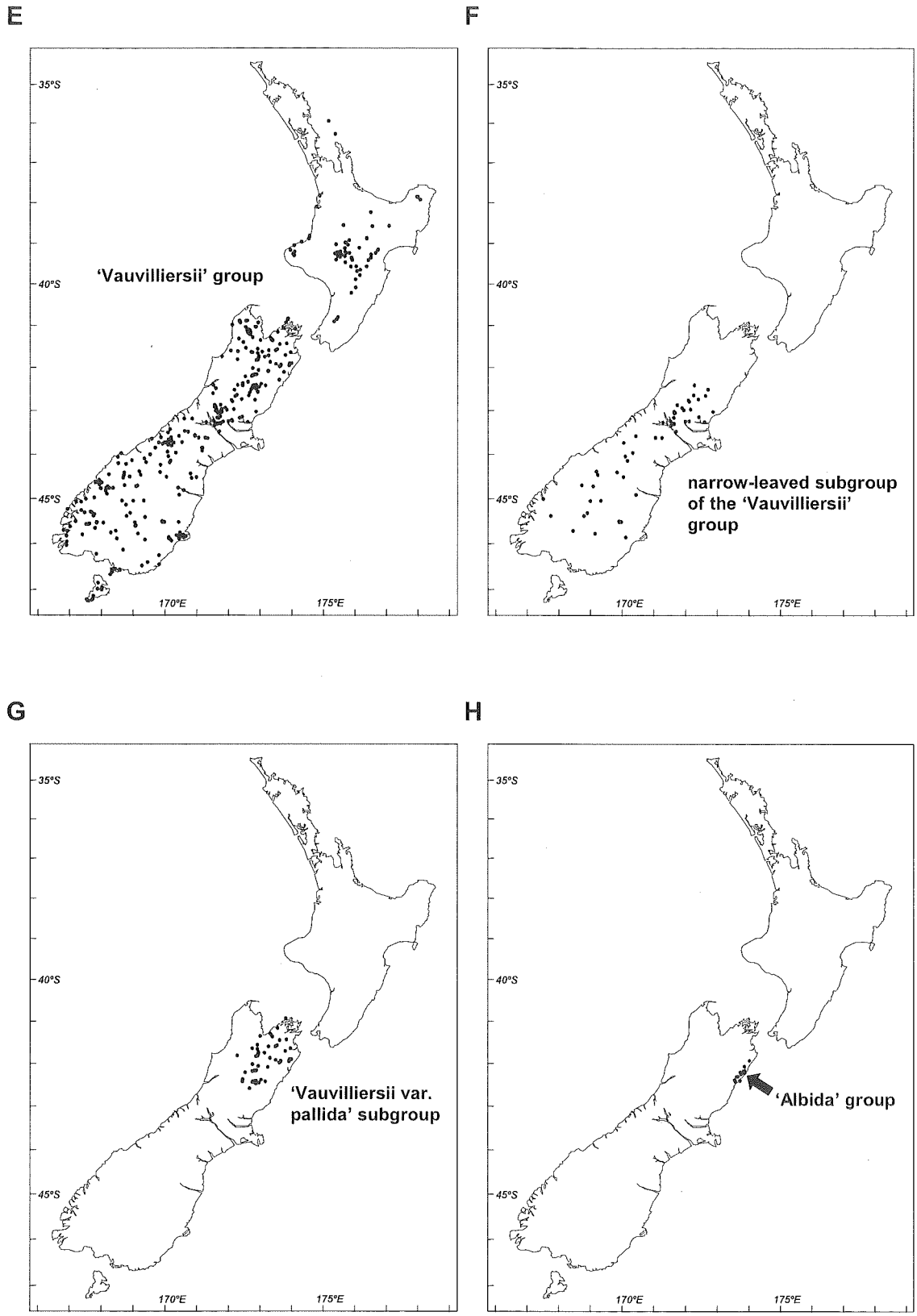


Fig. 5.1 Distributions. **A**, 'Amoena' group; **B**, 'Retorta' group; **C**, 'Leptophylla' group; **D**, 'Fulvida' group; **E**, 'Vauvilliersii group; **F**, narrow-leaved subgroup of the 'Vauvilliersii' group; **G**, 'Vauvilliersii var. pallida' subgroup; **H**, 'Albida' group.

Clinal variation and hybrid zones

The groups recognised as a result of this study exhibit a more or less continuous distribution. In the widespread groups, occupying large areas without a major geographic barrier, there is often complex geographic variation in which several characters vary simultaneously in clinal patterns.

The 'Fulvida' group shows variation within floral counts and leaf size from east to west of its range, with floral counts from small to large and leaves from narrow to broad (within the character range for this taxon). There is also a north-south variation within this group. The absolute leaf length and the ratio of leaf length to width increase with increasing latitude. The northern populations of the 'Fulvida' group show higher floral counts than the population further south in its range of distribution.

Throughout the distribution of the 'Leptophylla' group there appears to be a gradual change in habit from north to south. In northern populations, the habit of the plants is very lax, the upright branches commonly reflexed. Populations found on true sand dunes tend to be prostrate, thus reflecting the habit of the more northerly 'Retorta' group. Tomentum colour (colour and density of exudates) varies from greyish white to greyish yellow on the east coast of the North Island.

The 'Vauvilliersii' group, the group with the largest range, is the most polymorphic group and shows considerable geographic variation (which is maintained under cultivation). Exudate colour and density vary considerably between populations of this group. The habit ranges from very tall upright shrubs with large leaves from lower altitudes in the southern part of its range, to low, almost prostrate shrubs with slightly smaller leaves on mountain ridges in the more northern parts. The latter have been previously recognised as a distinct taxon, *Cassinia fulvida* var. *montana* Allan as a part of C. *fulvida*.

Hybrid zones are geographic areas where two (or sometimes more) taxa are in contact which each other and interbreed. Generally, outside a hybrid zone, the two or sometimes more taxa retain distinct differences from one another. In the case of *Ozothamnus leptophyllus* such forms are treated as distinct groups. The limits of hybrids must be taken into account when assessing a taxonomic treatment, especially where more or less fertile hybrids commonly occur between some distinct forms (Davis 1978).

Herbarium studies as well as observations in the field (Appendix 3) have verified that all recognised groups of *Ozothamnus leptophyllus* produce putative hybrids where their geographical ranges overlap. The flowering times for representatives of the different groups are identical or largely overlapping (Section 1.4.2), providing opportunity for hybridisation.

Gene exchange between different populations of *Ozothamnus leptophyllus* is likely to have occurred extensively over the last 1000 years since Maori and European settlers have modified the environment. Such phenomena are considered to have influenced species' distributions and increased opportunities for gene exchange in *Chionochloa* (Molloy et al. 1963, Connor 1967) and *Sophora* (Heenan et al. 2001). It is very probable that the periodic firing and heavy grazing practised in certain areas, producing a disturbed environment, have contributed significantly to creating conditions conducive to hybridisation (Section 1.3.2).

In the far north of its range, in the vicinity of the coastal cliffs of Kerr Point and Surville Cliffs, the 'Retorta' group is seen to cross with the 'Amoena' group. The study of herbarium specimens supported Carse's (1930) observation that there are three forms (H. Carse, near North Cape, Northland, "The Carse Herbarium", no. 1589a/5, CHR 333803; H. Carse, near North Cape, Northland, "The Carse Herbarium", no. 1589a, CHR 333805), one sharing most character states with the 'Amoena' group, but with shorter, more yellow leaves and broader capitula, a second form also similar to the 'Amoena' group, but with even smaller leaves, compared to the first form, and a third form, which resembles members of the 'Retorta' group, but with few florets in smaller capitula. Examples of putative hybrids between 'Retorta' and 'Amoena' at the North Cape are CHR 10334 "The Carse Herbarium", no. 1584/11, collector: H. Carse; CHR 10335 "The Carse Herbarium", no. 1584/10, collector: H. Carse; CHR 10333, collector: H. Carse; WELT 58307, collector: H. B. Matthews; CANU 11072, collector: M. T. Kalin, no. 67322; OTA 002440, collector: G. T. S. Baylis; OTA 002441, collector: G. T. S. Baylis and AK 229535, collector: P. J. de Lange.

Specimens combining characters from the 'Retorta' and 'Vauvilliersii' groups have been collected from several, mostly coastal, localities in the North Island. On the coast near and south of New Plymouth, several specimens have been found with large wide leaves and small corymbs with large capitula (e.g. G. W. Mason, Tongaporutu, Taranaki, NZFRI

4128), as well as plants with small, densely set leaves and large corymbs with small capitula (e.g. C. C. Ogle, White Cliffs, Waipiugau Stream, CHR 208893). A specimen intermediate in both floral and vegetative characters has been collected by D. Petrie in Paritutu, New Plymouth, Taranaki (CANU 4615). Near the Te Toto Stream, northwest of Mount Karioi, South Auckland Land District, A. P. Druce collected a specimen that appears intermediate between the 'Vauvilliersii' and 'Retorta' groups (CHR 262214). Polymorphic populations with representatives of both groups and intermediate plants were collected in the Bay of Plenty (e.g. Mt Maunganui, W. R. B. Oliver: WELT 58463, WELT 58470, WELT 58468) and Great Barrier Island (e.g. T. Kirk, WELT 58329: 4 specimens on sheet, two clearly 'Vauvilliersii', one intermediate and one 'Retorta'). Other examples of putative hybrids from Great Barrier Island are "The Carse Herbarium", no. 1584/4, CHR 10341; R. C. Cooper, Kaitoke Beach, AK 104048; B. D. Clarkson, Medlands Beach, NZFRI 19277. Herbarium studies have revealed that putative hybrids between the 'Retorta' and 'Vauvilliersii' groups are quite common, while intermediate forms between the 'Leptophylla' and 'Vauvilliersii' groups have been less frequently collected as seen in herbarium collections. However some putative hybrids occur in the North and the South Island between these two groups and are characterised by a compact habit, large, yellowish leaves (characteristic of the 'Vauvilliersii' group) and large, spreading corymbs with many small capitula (characteristic of the 'Leptophylla' group) (e.g. Te toto Gorge Track, Mt Karioi, South Auckland Land District, B. D. Clarkson, NZFRI 18677; near Mangahume Stream, Egmont Coast, Taranaki Land District, A. P. Druce, CHR 131041; near Cape Egmont, Egmont Coast, Taranaki Land District, A. P. Druce, CHR 246054; Tararua Range, Wellington Land District, J. M. Ward, no. 671155, CANU 29911; Hokio near Levin, Wellington Land District, F. C. Duguid, CHR 385318; East Cape Lighthouse, Gisborne Land District, M. Heginbotham, CHR 370910; roadside below Bald Spur, D'Urville Island, Marlborough Land District, A. P. Druce, CHR 387221; road to Collingwood, near Parapara River, Nelson Land District, G. Brownlie, CHR 377144).

In the north of the 'Leptophylla' group range, where it overlaps with the 'Retorta' group, the presence of specimens with smaller, less spreading panicles and larger capitula suggest that these two groups may be hybridising. Intermediate specimens have been collected from coastal areas of the South Auckland Land District (e.g. Waikawau Beach, A. P. Druce, CHR 208879), the Taranaki Land District (e.g. Moutoti Stream, near Oaonui,

A. P. Druce, CHR 245937; Puketapu Road, A. P. Druce, CHR 245991; near Cape Egmont, A. P. Druce, CHR 246054), the Gisborne Land District (e.g. Maraenui near Opotiki, A. E. Esler, no. 3380, CHR 225401; Whanarua Bay, M. Heginbotham, CHR 370912; Maraenui Hill, M. Heginbotham, CHR 370915; Parikanapa Road, Ngatapa, B. D. Clarkson, NZFRI 18449), and the Hawke's Bay Land District (e.g. near Tangoio north of Napier, B. Donovan, CHR 216208; Aropaoanui Beach, M. J. A. Simpson & R. B. Allen, CHR 404220).

Putative hybrids between members of the 'Leptophylla' and 'Fulvida' groups occur in the South Island. Polymorphic populations of these groups are very common in coastal habitats in the Marlborough Land District from Cloudy Bay to the Flaxbourne River, where the geographical ranges of the two groups overlap. These populations contain intermediate and pure coloured (greyish green, yellowish green) individuals. Many character combinations are present, which may represent re-combinations of high floret number, high receptacle scale number and larger capitula in individuals with no, or less, yellow exudates (as seen in the 'Leptophylla' group) and the reciprocal combination in yellow individuals (representatives of the 'Fulvida' group). Herbarium studies confirmed these observations. Putative hybrids have been collected for example from the following locations in coastal and lowland areas in the Marlborough Land District: Weld Pass, north-east Marlborough, B. H. Macmillan, CHR 155435; Parikawa, South Marlborough, G. Brownlie, Herbarium G. Brownlie, no. 518 and 519, CHR 377142 and CHR 377143; Ward Beach, near Flaxbourne River mouth, I. Breitwieser, no. 784, CANU 3370.

During this study, very large polymorphic populations containing putative hybrids were found further inland in Marlborough, where the clearing of native vegetation extended the habitat of *Ozothamnus leptophyllus*. It can be hypothesised that when the natural barrier provided by dense vegetation was removed, the 'Leptophylla', 'Fulvida', 'Vauvilliersii' and 'Albida' groups extended their distributions and finally met and interbred, leading to a breakdown of the differences that might be used to characterise them in their more natural and isolated localities.

Members of the 'Albida' group seem to frequently hybridise with those of the typical 'Vauvilliersii' and 'Vauvilliersii var. pallida' groups, as indicated by intermediate forms encountered during this study (Section 2.3.3.1, Section 2.4.4, herbarium studies). Hybrids between *Cassinia vauvilliersii* and *C. albida* were reported by Cockayne and Allan

(1934). Due to the limited range of the 'Albida' group these putative hybrids can be found only in the vicinity of the Seaward Kaikoura Range. Hybrids between the 'Albida' and 'Vauvilliersii var. pallida' groups seems to occur occasionally throughout the Kaikoura Ranges and their vicinities in spite of the absence of one parental group (e.g. Mount Benmore, north of Kekerengu, A. P. Druce, CHR 249188; Chalk Range, near Brian Boru, A. P. Druce, CHR 274833). Putative hybrids between these two groups have been also collected further inland in Marlborough (e.g. east of Taylor Pass, A. P. Druce, CHR 311873), Nelson (e.g. Whekeri, W. Martin, WELT 58278; Black Hill, Lake Rotoiti, M. J. A. Simpson, no. 3175, CHR 125350; Lake Rotoiti, G. Brownlie, no. 337, CHR 377146; Golden Downs Forest, OTA 002422; Mt Robert Skifield Road, I. Schönberger, no. 65A, CANU 38575) and North Canterbury (e.g. beyond Jacks Pass, G. Brownlie, no.729, CHR 377145).

At lower altitudes in the Kaikoura Ranges, where the coastal 'Fulvida' group extends inland, intermediate forms between the 'Fulvida' and 'Albida' groups were recorded (Section 2.4.4). They occur usually in polymorphic populations in which some specimens resemble one or other of the groups, but most have intermediate status by combining character states of the different groups. Such intermediate specimens have been collected from following localities in Marlborough: Wandle Bush, Nelson, Herbarium Cockayne, WELT 58215; Hapuku River, NZFRI 15338; Mount Fyffe, E. Edgar, CHR 127073 and CHR 127075; Puhi Puhi Stream, tributary of Hapuku River, E. Edgar, CHR 127074; Kowhai Bush, A. T. Dobson, CANU 019720; Blue Duck Reserve, Kaikoura, D. A. Norton, CANU 36797; Mt Fyffe, A. T. Dobson, CANU 018151.

Examination of herbarium specimens indicated that in Nelson, Marlborough and North Canterbury, representatives of the 'Vauvilliersii var. pallida' subgroup may interbreed with typical representatives of the 'Vauvilliersii' group, and at lower altitudes with representatives of the 'Fulvida' group. The putative hybrids show varying degrees of yellow exudates, glutinosity and leaf shapes and sizes. Populations containing representatives of both groups, and specimens with intermediate appearance, have been found on Dun Mountain and along the Mineral Belt, Nelson Land District (e.g. Dun Saddle, J. F. F. Hobbs, NZFRI 23142; Dun Mountain, J. A. Petterson, CHR 77921; upper Maitui Valley, W. R. B. Oliver, WELT 9633; Mineral Belt, V. D. Zotov, CHR 3930), Tableland, Nelson Land District (e.g. F. G. Gibbs, CHR 140783), Jollies Pass, North

Canterbury (e.g. V. D. Zotov, CHR 20532), Twenty-four Tarn Basin, Herbert Range, Nelson Land District (e.g. A. P. Druce, CHR 395965), West Amuri, Hope River, Canterbury Land District (e.g. W. B. Brockie, CHR 222003), Island Saddle, Marlborough Land District (e.g. A. D. Wilton, CANU 37788) and several other localities. Polymorphic populations including representatives of the 'Vauvilliersii var. pallida' subgroup, the 'Leptophylla' and 'Fulvida' groups, have been encountered during this study on and below the Chalk Range, the Isis Stream valley in Marlborough (Section 2.4.4) and other localities in Marlborough, for example, the Brancott Station north-west of Blenheim (see also Appendix 3).

In the south of its range, the 'Vauvilliersii var. pallida' subgroup may cross with the narrow-leaved subgroup of the 'Vauvilliersii' group. Some intermediate-looking specimens with narrow, but less fulvous leaves, have been collected in North Canterbury at the Doubtful River, West Amuri Range (W. B. Brockie, CHR 222232) and in Williams Valley, Mount St Patrick (A. P. Druce, CHR 275312).

Putative hybrids between the typical 'Vauvilliersii' and the 'Fulvida' groups occur frequently in Otago where the 'Vauvilliersii' group extends its range down to sea level and representatives of the 'Fulvida' group can be found at higher altitudes well inland in Central Otago. The previously described *Cassinia fulvida* var. *linearis* from Dunedin, placed in the 'Fulvida' group in this study, may be of hybrid origin since its leaf length is at the very top of the range for the 'Fulvida' group. Herbarium studies revealed several localities in the Dunedin area (e.g. Dunedin, Petichet Bay, Flagstaff Hill) where representatives of the 'Vauvilliersii' and 'Fulvida' groups, and intermediate forms, occur together. Further inland in Central Otago and Southland, the 'Fulvida' group is quite rare and therefore putative hybrids and mixed populations can be found only occasionally (e.g. Mount Koinga, Dunstan Range, Otago Land District, W. G. Lee, CHR 320488; south of Lake Ohau, Southland Land District, P. N. Johnson, CHR 320218; Tinwald Creek, Mount Pisa, Otago Land District, V. D. Zotov, CHR 94060).

In Canterbury and North Otago, populations containing representatives of both the 'Vauvilliersii' and 'Fulvida' groups, and/or intermediate forms are scarce. Field observations suggest that these two groups are ecologically separated and occupy different habitats. The 'Fulvida' group occurs mainly in indigenous-induced, heavily grazed shrub- and grassland, while the 'Vauvilliersii' group occupies more "primeval" (without

permanent human habitation or modification) habitats where it represents a part of natural plant communities such as *Hebe* scrub, shrub-composite scrub, *Dracophyllum* scrub and herb-moor communities (Section 1.3). However, both groups and putative hybrids seem to be present in the Mount Cook area (e.g. Old Man Scarp, Balmoral Station, West of Lake Tekapo, B. H. Macmillan, CHR 166041; the Hermitage, Mt Cook, H. D. Wilson, CHR 254086).

Despite large overlapping ranges (Fig. 5.1 D and F), putative hybrids between the narrow-leaved subgroup of the 'Vauvilliersii' group and the 'Fulvida' group are very rare and only very few examples could be found from Otago and Canterbury by assessing herbarium specimens (e.g. Wilderness Reserve, B. Molloy, CHR 386773; Horse Range road, J. Glasson, no. 129, CHR 438147; Craigieburn Stream, T. McIntosh, CANU 25390 and CANU 25223). These groups seem to be also ecologically separated, with members of the narrow-leaved subgroup of the 'Vauvilliersii' group replacing the typical representatives of the 'Vauvilliersii' group in habitats without permanent human habitation or modification and representatives of the 'Fulvida' group occurring in coastal and lowland habitats and in "indigenous-induced" communities of the lowland and low-montane belt.

Representatives of the narrow-leaved subgroup of the 'Vauvilliersii' group seem to cross occasionally with typical representatives of the 'Vauvilliersii' group (indicated by intermediate leaf sizes) throughout the range of the both groups (e.g. Slopes of The Hooligan, Upper Hurunui River, Canterbury Land District, M. J. A. Simpson, CHR 176752; Old Dunston Road, Maniototo, Otago Land District, P. Wardle, CHR 279958; south-east Spur, Fog Peak, Torlesse Range, Canterbury Land District, W. R. B. Oliver, CHR 290762; Mount Somers, Canterbury Land District, A. P. Druce, CHR 402045; tarn at East side of Mt. Misery, south-west of Poulter Hill, Canterbury Land District, D. R. Given, CHR 405302; Nevis Valley, Otago Land District, A. P. Druce, no. APD1368, CHR 471960). However, in areas where both groups are present the 'Vauvilliersii' group seems to occupy higher altitudes compared to the narrow-leaved subgroup of the 'Vauvilliersii' group. An example can be found in the Cass area, Canterbury, where representatives of the 'Vauvilliersii' have been found only on mountain tops surrounding the Cass Basin (e.g. Sugarloaf, Cass, N. R. Foy, CHR 108638; Woolshed Hill, Cass, J. M. Ward, no.s 67369 and 67370, CANU 26810; Mt Horrible, Cass, J. M. Ward, no.s 65161 and 65162, CANU 26813 and CANU 26812).

Although the occurrence of hybridisation seems to obscure the boundaries between the different *Ozothamnus leptophyllus* groups, it may have been responsible for giving rise to the now stable entities recognised as subgroups within the 'Vauvilliersii' group. For instance the narrow-leaved subgroup may have arisen as a hybrid between the 'Vauvilliersii' and 'Fulvida' groups. The results of the numerical phenetic analyses support this hypothesis by indicating the phenetic intermediacy of representatives of this subgroup (Section 2.3.3). Similarly, it is possible that the 'Vauvilliersii var. pallida' subgroup may have originated as hybrid populations between the 'Vauvilliersii' and 'Albida' groups.

Concepts of species and infraspecific categories

Before a new classification is introduced, the taxonomic concepts involved in the intended changes to classification of the *Ozothamnus leptophyllus* complex will be discussed.

Species concepts

The complex population system of *Ozothamnus leptophyllus* is not directly comparable to genetically discrete species in other genera. However, phenetic grouping of *O. leptophyllus* populations based on morphological and genetic criteria led to the recognition of distinct groups.

Based on the available data and the methods of analysis used here, the 'numerical phenetic species concept' (Crovello 1970, Sokal 1973), using morphological and genetic distances, is most appropriately applied here. This species concept includes the morphological species concept, the classical phenetic species concept (Sokal 1973), as well as the genetic species concept *sensu* Stuessy (1990).

The morphological species concept is probably the most frequently employed species concept, especially by revisionary workers or herbarium and museum taxonomists (Stuessy 1990). Morphological similarity (or dissimilarity) is the sole criterion for determining species. Thus, the degree of individual morphological similarity or difference (vs. distinctiveness) is the decisive criterion of species status. Species identified under this concept and solely on the basis of morphological differences are called morphological species or morphospecies. The 'classical phenetic species concept' is often used as a synonym for the morphological species concept (Sokal 1973). The degree of morphological differentiation and similarity is numerically assessed and a multivariate morphospace occupied by a given species is defined. The morphological species concept is often criticised as overlooking the secondary role of morphological differences in species formation. Morphological differences among species are a secondary by-product of genetic divergence and not the cause of it. Heywood (1967) and Stuessy (1990) replied to this criticism, stating that it does not matter how the discontinuities have arisen and if they represent actual biotypes or not. If discontinuities exist, taxonomic units will be recognised accordingly. It is likely that morphological discontinuities represent biological limits of

isolation, commonality of interbreeding and genetic divergence (Stuessy 1972). Therefore, reproductive isolation is also implied by the morphological species concept. The morphological species concept has worked well even in those cases where intermediates and hybridisation are known (Burger 1975), and from a practical standpoint in the preparation of floras, the circumscription of species based upon easily observable morphological features is a most sensible approach.

The genetic species concept, according to Stuessy (1990), defines species by a measure of genetic differences or distance among populations or groups of populations. As in the morphological species concept, numerical phenetic methods are used to assess these differences. This more recent species concept is closely related to the biological species concept. Both genetic and biological species concepts attempt to identify the underlying biological "reality" of species. According to the biological species concept, a species is a group of interbreeding populations, which are reproductively isolated from other such groups (Mayr 1969). The biological species concept is based on the idea that if gene flow can occur between groups (because they can reproduce and produce healthy, fertile offspring) then these groups can affect each other genetically and should be grouped into a single species. The genetic species concept assumes that the biological factors of gene flow and reproductive isolation are operative and that both are reflected in the genetic differences or distance among populations or groups of populations. Given that there are abundant genetic data available, a genetic version of the numerical phenetic species concept can be used (called by Mallet (1995) the genotypic cluster definition). Genotypic clusters can be identified by the presence of gaps between groups of multilocus genotypes within a local area, in the same way that morphological cluster species are identified by morphological gaps. Genotypic cluster species are very similar to the practical taxonomic application of the biological species concept but the genetic species concept emphasises genetic rather than reproductive factors which are responsible for keeping population systems isolated (Bock 1986). If the genetic basis of all the species was known it might be possible to apply a genetic characterisation of a species taxon (Stebbins 1950).

Species as genotypic clusters are easier to use in taxonomy than species based on idealised evolutionary or biological concepts, because knowledge of biological and evolutionary processes are not required prior to the study (Mallet 1995). Despite the

increased ability of molecular techniques over the last two decades to assess genetic divergence, data are not yet available to indicate general levels of genetic divergence for each of the levels of taxonomic hierarchy in plants, and they may never be fully meaningful, even if available (Stuessy 1990).

The ecological species concept (Van Valen 1976, Andersson 1990) may also contribute to resolution of taxonomic problems in *Ozothamnus leptophyllus*. This concept advocates closely examining morphological variation and correlating this with different adaptive zones. The ecological species concept states that a species throughout its range occupies a single niche. Because of this, all individuals of a species experience the same selection pressures and this maintains uniformity in the species. The ecological species concept is mentioned by Stuessy (1990) as one of the biosystematic species concepts. Raven and Raven (1976) encountered very diverse habitats of New Zealand *Epilobium* species and believed that uniform habitat selection pressures were maintaining species. They were adopting the ecological species concept even before it has been discussed and outlined by Van Valen (1976). Heenan et al. (2001) could show the utility of the ecological species concept among closely related, and often difficult to discriminate, sibling species within *Sophora* in New Zealand. The extensive field observation and experimental cultivation carried out in this present study justify the application of this species concept in addition to the 'numerical phenetic species concept'.

The results of the morphological studies of the *Ozothamnus leptophyllus* complex indicate the presence of hybrids and intermediate forms between distinct entities, which would correspond with Grant's (1957) definition of a 'syngameon' as "the sum total of species or semispecies linked by frequent or occasional hybridization in nature; a hybridizing group of species; the most inclusive interbreeding population" (Grant 1957:67). If the term 'semispecies' is applied to the different entities within the *O. leptophyllus* complex it has to be used in the sense of geographic segregates of a good species, which are so morphologically distinct as to be treated almost as a distinct species (Mayr 1940).

Infraspecific categories

Although the species is commonly regarded as the lowest recognisable rank which is essential for 'general taxonomic purpose' (Stace 1989), there is often the need to express infraspecific variation in taxonomic terms, particularly in situations in which complex patterns of variation occur (Stuessy 1990). The use of infraspecific ranks can prevent the 'over-splitting' of species. Stace (1989) gives several examples where the lack of means to express infraspecific variation led to the recognition of "too many species", for example, in the Flora U.R.S.S. (1934-1964) in which no infraspecific taxa are recognised.

The use of infraspecific categories has varied over the years and in different places (see Stuessy (1990) for a summary of the history of the use of infraspecific categories). Remaining is the problem of which and how many infraspecific rank(s) to use. In particular, the use of subspecies and variety has been the cause of considerable confusion. In fact, there are no generally acceptable distinctions in usage of the subspecific and varietal rank (Davis 1978). Codes of Nomenclature give no rules or recommendations on how to use the infraspecific categories, and in many Floras they are used without comment, with a species divided into one or the other categories seemingly at random. Davis (1978) recommended that taxonomists creating infra-specific taxa or changing ranks indicate the way in which they use the rank.

In this study, Stuessy's (1990) recommendations are followed. He recommended that both categories, subspecies and variety should be available for use. Several criteria are utilised in this study including morphological distinctness, geographical cohesiveness and genetic divergence, as well as the presence of natural hybrids.

Geography is a very important, if not the most important, component in the recognition of infraspecific taxa (Stuessy 1990). Subspecies and varieties are considered only if the distributions are largely allopatric. Morphologically distinct population systems that are geographically completely overlapping are reproductively isolated and best viewed as different species. Subspecific recognition may be appropriate for partly sympatric, partly intergrading, entities as shown by Raven and Raven (1976). The subspecies is usually considered to be a geographically defined taxon (Burger 1975). Most commonly, morphologically distinguishable regional races are recognised as subspecies, with or without some degree of morphological overlap (Davis 1978). Babcock (1947) defined

subspecies as "an intraspecific population with a geographic distribution, which is partly isolated from that of the rest of the species but which overlaps more or less the distribution of one or more other subspecies".

Morphologically distinct local populations within the range of a subspecies are often treated as varieties (Davis 1978, Stuessy 1990). Davis (1978) warned against the use of the varietal rank for striking variants without knowing their distribution, which may easily be modifications that should not be formally named. Small, distinctive, local races that grow outside the range of the regional and distinctive subspecies should not be given varietal rank, but treated as subspecies. However, if a variant forms local populations scattered throughout the range of a species or subspecies it might be given varietal rank (Davis 1978). Davis (1978) mentioned that regional subspecies are not necessarily better distinguished morphologically than local varieties and that it is more a matter of distribution pattern as to which rank is used.

The genetic criterion forms an important part of the evaluation of infraspecific patterns of variation (Stuessy 1990). Unlike species, subspecies are expected to have gene exchange and 'one would expect a "genetic yardstick" in which subspecies would be more genetically distinct from each other than would be varieties' (Stuessy 1990).

In many instances one might be faced with reduced genetic divergence but with morphogeographic compartmentalisation, indicating recent diverging from a common evolutionary origin (Vijverberg 2001). If this is the case, genetic data would not be helpful for differentiating infraspecific taxa. However in cases such as the present study, where genetic divergence is congruent with morphological divergence, genetic data can be extremely helpful in such differentiation. If several genes are involved in the discrimination of an ecological race, the rank of subspecies has often been used (e.g., Clausen 1951, *Geranium robertianum* ssp. *maritimum*). Consequently, ecological races that differ less markedly might be called varieties (e.g., Clausen 1951, *Geranium sanguineum* var. *prostratum*).

Taxonomic concepts applied to *Ozothamnus leptophyllus*

Application of the above species concepts results in accepting *Ozothamnus leptophyllus* as a single species. The degree of separation based on morphological and genetic data is considered insufficient to describe distinct entities as species. This is illustrated by the low phenetic differences (Section 2.3.3) or 'gaps' among groups. Distinct entities are found to hybridise freely and no evidence is found suggesting reproductive isolation (personal observations, Section 2.4.4; Cockayne 1928, Carse 1930, Cockayne & Allan 1934, Kalin 1967a). Limited phenetic discontinuity among the groups distinguished in this study is likely to be due to partial allopatry.

There remains the question of how best to classify the variation within *Ozothamnus leptophyllus*. The most practical way to express this variation pattern seems to be by adopting a three-level hierarchy, accepting *O. leptophyllus* as a single species and using two infraspecific categories, subspecies and variety, to describe infraspecific variation. The use of quadrinomials in the New Zealand flora has been limited, e.g., *Chinochloa rubra* (Connor 1991). The category of variety was applied by Allan (1961) to New Zealand *Cassinia* to account for various patterns of morphological and geographic variation within what were then accepted as species. Three of the five species of *Cassinia* accepted by Allan (1961) were subdivided into varieties. However, Allan (1961) generally did not use the subspecies category and usually only recognised varieties.

New Zealand's present foremost taxonomists often prefer to use the rank of subspecies as the sole infraspecific rank (Garnock-Jones (1980) in *Parahebe*, Edgar (1986) in *Poa*, Garnock-Jones (1986) in *Myosurus*, Webb (1987) in *Senecio*, and Sykes (1992) in *Macropiper*). Lloyd (1972) in *Cotula* section *Leptinella* and Connor (1991) in *Chionochloa*, used both subspecies and variety. It should be stated that Lloyd did not generally use the rank of variety and was only inclined to do so on this occasion to accommodate a particularly striking variant. He treated geographical variants as subspecies, and entities that could be defined by at least one character, not including geography, as a distinct variety.

The subspecies category in this study is employed for major segments of a species which are morphologically distinguishable from each other and have their own

geographical area, with some overlap and interbreeding at the margins. In this study most subspecies are also defined based on genetic information derived from AFLP analyses.

No evidence could be found that was suffient to reject the null hypothesis that all subgroups comprised a single species. In total six distinct groups are assigned as subspecies status within *Ozothamnus leptophyllus*. The 'Amoena', 'Albida', 'Retorta' and 'Vauvilliersii' groups have very similar high levels of overall distinctness if both molecular and morphological data are considered. The 'Albida' group appears to be closely allied to the 'Amoena' group if only morphological data are considered. They share several characters such as leaf size and shape, lack of yellow exudates, and the size of many floral parts, but are distinct in habit, and number of florets and receptacle scales. AFLP data clearly separates these two groups.

Molecular data fail to differentiate clearly between the 'Albida' and 'Vauvilliersii' groups, but morphological data provide a much clearer distinction (Section 2.3.3). The lack of yellow exudates, a very dense tomentum on the abaxial surface of the leaves and branchlets, larger leaves, thick pappus hairs that are not much spreading at the tips and short basal appendages of the anthers separate the 'Albida' group from the 'Vauvilliersii' group (Section 2.4.1).

The 'Fulvida' and the 'Leptophylla' groups are quite similar overall (genetically and morphologically), with only a few morphological characters distinguishing between them. Colour differences in their leaf and branchlet tomentum due to differences in tomentum density, exudate density and colour, differences in floral counts and receptacle scale number do distinguish these groups. In addition to the genetic and morphological distinctiveness, the distribution of these groups contribute largely to the decision to recognise them as subspecies. The distribution of the 'Leptophylla' group ranges from latitude 37°30'S to 42°S, while the 'Fulvida' group extends from latitude 41°30'S southwards.

Most of the different groups are naturally found in distinct geographical areas, which overlap at the edges with one ore more groups (Fig. 5.1). The 'Fulvida' and the 'Leptophylla' groups, even if less distinct from each other overall, compared with any other subspecies pair, are designated subspecies primarily because they clearly fulfil the distribution criteria for subspecies. Neither the 'Fulvida' nor the 'Leptophylla' group can

be seen as a local population within the geographical range of the other, as might apply to varieties, even if their ranges overlap considerably.

Morphologically distinct entities within the range of a subspecies are designated varieties. Such classification can be useful for describing hierarchical patterns of variation. While it may be viewed as cumbersome, in practical terms such a classification need not be any more cumbersome than any other infraspecific classification, since in common usage the names of the subordinate taxa can be given simply as *O. leptophyllus* var *vauvilliersii*, *O. leptophyllus* var. *collinus*, and *O. leptophyllus* var. *pallidus* (ICBN Ch. 3, Sec. 5, Art. 24.1; Greuter et al. 2000). Full details of the classification (e.g., *O. leptophyllus* subsp. *vauvilliersii* var. *pallidus*) need only be used when desired. The distinction of varieties in this study is based on a single, marked, constant character difference. Two segregate varieties are accepted within the 'Vauvilliersii' group. The first is instantly recognisable by narrower leaves compared to that of the rest of this subspecies. The second subgroup is the 'Vauvilliersii var. pallida' group that is distinguished by the pale leaf and branchlet colour due to the lack of yellow coloured exudates.

Revision of *Ozothamnus leptophyllus*

Typification within *Ozothamnus leptophyllus*

A type element is one that is permanently associated with a given scientific name. The type of the name of a species or infraspecific taxon is a preserved specimen or an illustration. Each name should have only a single type specimen that indicates to what taxon the name should apply.

The rules and practice of typification have changed over the years. The current rules and recommendations are detailed in the International Code of Botanical Nomenclature (ICBN), Chapter 2, Section 2, Articles 7-10 (Greuter et al. 2000). There are six main categories of types recognised under the International Code of Botanical Nomenclature (ICBN Ch. 2, Sec. 2, Art. 9):

Holotype: The single specimen designated as the type of a name of a species, or infraspecific taxa, by the original author at the time the species name and description was published. For older names (prior to 1958, ICBN Ch. 4, Sec. 2, Art. 37.1) based on a single specimen, the holotype need not have been explicitly designated "type" by the publishing author.

Lectotype: A specimen chosen by a later researcher to serve as the primary type. It is chosen from among the specimens available to the original author of a name when the holotype was either lost or destroyed, or when no holotype was designated. Isolectotypes are duplicates of a lectotype.

Isotype: A duplicate specimen of the holotype.

Syntype: Any of two or more specimens listed in the original description of a taxon when a holotype was not designated. Isosyntypes are duplicates of a syntype.

Paratype: a specimen cited in the protologue that is neither the holotype, isotype, nor one of the syntypes.

Neotype: A specimen chosen by a later researcher to serve in place of a holotype when all specimens available to the original author of a name have been lost or destroyed.

Epitype: "a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically

identified for purposes of the precise application of the name of a taxon." (ICBN Ch. 2, Sec. 2, Art. 9.7). The holotype, lectotype, or neotype that the epitype supports must be explicitly cited when the epitype is designated (Art. 9.18).
Additional to the above types, Brummitt (1985) defined the **lectoparatype**: if a syntype is designated as lectotype, the other syntypes become lectoparatypes.

Prior to 1 January 1958 (ICBN Ch. 4, Sec. 2, Art. 37) the publication of the new name did not require the designation of a type. References to type specimens in early plant systematics literature are often absent or vague.

Names within *Ozothamnus* (as *Cassinia*) in New Zealand have been published intermittently since 1786. For many of the early names no holotype exists. Before taxa within *Ozothamnus leptophyllus* can be named, it is necessary for the types of all names published in *Cassinia* to be checked and if necessary further typification has to be undertaken.

Specimens cited in the protologues associated with the basionyms for *Ozothamnus leptophyllus* and all names of taxa now included in *O. leptophyllus* were examined from the following herbaria: Muséum National d'Histoire Naturelle France, Paris (P); Department of Natural History of the British Museum, London (BM); Royal Botanic Gardens, Kew, London (K); Allan Herbarium, Landcare Research, Lincoln, New Zealand (CHR); Auckland War Memorial Museum, Auckland, New Zealand (AK); Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand (WELT).
Appendix 1 Table 2 lists the viewed and examined type material with all the information given on the herbarium sheets.

The following typifications are made according to the ICBN, Chapter 2, Section 2, Articles 7-10 (Greuter et al. 2000). Examination and assessment of the material held at WELT was undertaken by J. M. Fox, collection manager in the Museum of New Zealand Te Papa Tongarewa, Wellington in 2000 and is here cited with permission. Homotypic synonyms are indicated by ≡, heterotypic synonyms by =. Abbreviations of periodicals follow Lawrence et al. (1968), of books Stafleu and Cowan (1976, 1979, 1983), and of names of authors Brummitt and Powell (1992).

Calea leptophylla G.Forst., Prodromus: 55 (1786)

≡ *Cassinia leptophylla* (G.Forst.) R.Br., Trans. Linn. Soc. Lond. 12: 126 (1817).

TYPE COLLECTION: "in New Zeeland" (Forster 1786), "Prope Queen Charlotte's Sound" (Brown 1817).

LECTOTYPE: The Forster Herb. sheet held at Kew (photograph seen) (*fide* Allan 1961: 727), fruiting piece on left-hand side of sheet (here designated).

LECTOPARATYPES: The Forster Herb. sheet held at Kew, right-hand side piece, centre piece; Forster Herb., BM 602391!, Herb. Pallas ex Forster's Herb., BM 602392!

Notes: There are at least three herbarium sheets of *Calea leptophylla*, collected by G. Forster. Forster did not specify a particular specimen as his nomenclatural type, and as none of his specimens are dated, it is not certain whether they represent a single collection event, or whether they originated from the same plant. Therefore lectotypification is necessary. One of Forster's herbarium sheets is held at Kew and contains three specimens, two non flowering and one fruiting. Allan (1961: 727) selected this sheet as the type but did not specify a particular specimen. The fruiting specimen is designated here as the lectotype. The remaining specimens on this sheet and the sheets in BM must be regarded as lectoparatypes.

The herbarium sheet held at Kew states locality, in Forster's handwriting, as "Habitat in New Zeeland". Brown (1817), when he changed the name to *Cassinia leptophylla* indicated as locality for Forster's specimens "Prope Queen Charlotte's Sound".

Cassinia amoena Cheeseman, Trans. & Proc. N.Z. Inst. 29: 391 (1897).

TYPE COLLECTION: North Cape, T. F. Cheeseman, January 1896.

LECTOTYPE: Cheeseman, Herbarium T. F. Cheeseman, AK 10298! (*fide* L. M. Cranwell) large piece on sheet (here designated).

ISOLECTOTYPES: Herbarium T. F. Cheeseman, AK 10299!; Herbarium T. Kirk ex Herb. T. F. Cheeseman, WELT 58304!

Notes: Cheeseman did not cite any specimens in his protologue. Therefore lectotypification is necessary. Each of Cheeseman's three herbarium sheets is dated "January 1896". In 1941 L. M. Cranwell selected the sheet AK 10298 as lectotype. This assessment was not published. Plate 107 of Cheeseman's "Illustrations of the New Zealand Flora" (1914)

depicts a drawing of *Cassinia amoena* which is most likely based on the the larger of the specimens on the selected lectotype. This is supported by a note in Cheeseman's handwriting on the label of AK 10298 and the obvious resemblance of this specimen with the illustration.

Cassinia albida* var. *canescens Cockayne, Trans. & Proc. N.Z. Inst. 38: 369 (1906)
≡ *Cassinia vauvilliersii* var. *canescens* (Cockayne) Allan, Flora N.Z. 1: 728 (1961)
TYPE COLLECTION: "L. Cockayne, Mt. Fyffe, 2000 ft."
HOLOTYPE: L. Cockayne Herbarium No. 9093, WELT 58358!

Notes: A herbarium sheet WELT 58279 (D. Petrie Herbarium; ex Cockayne Herbarium No. 39) presently in the type folder at WELT, includes three flowering pieces of *C. vauvilliersii* var. *canescens* which were collected by Cockayne on 10.02.1892 when he first visited Mt Fyffe, fourteen years before the name was published (Cockayne 1906). On the label of WELT 58279 Cockayne states: "*Cassinia* sp. new form to me but possibly *C. vauvilliersii*, if so very different to plants I have so named. Forming greater part of subalpine vegetation on Mt. Fyffe". However there is no indication on the label as to whether Cockayne was referring to *Cassinia albida* sens. str. or to *C. albida var. canescens*. J. Fox (2000, unpubl. information) recommends excluding this collection from the type folder "on the basis that it is uncertain as to whether is was available to Cockayne when he drew up the description of *Cassinia albida* var. *canescens*". WELT 58358 has been labelled by Cockayne "*Cassinia albida* (T.Kirk) Cockayne var. *canescens* Cockayne", and later annotated by him as the type. It was collected by Cockayne just prior to his publication of the name "*canescens*". Cockayne also noted on the label "as sent to Man. ed.2" which refers to "Manual of the New Zealand Flora, ed. 2" (Cheeseman 1925). J. Fox (2000, unpubl. information) suggests WELT 58358 as lectotype, but since Cockayne clearly chose this specimen to base his description on, it might be seen as the holotype for *C. vauvilliersii* var. *canescens.*

Cassinia fulvida var. linearis Kirk, Students' Flora: 315 (1899).

TYPE COLLECTION: "B. C. Aston, 02.02.1896, near Reservoir, Dunedin."

LECTOTYPE: T. Kirk Private Herbarium, WELT 58747/A! (here designated).

ISOLECTOTYPES: T. Kirk Private Herbarium, WELT 58747/B!; T. Kirk Private Herbarium, WELT 58748/A!; D. Petrie Herbarium, WELT 58665!; T. Kirk Private Herbarium, WELT 58748/B!; T. Kirk Private Herbarium, WELT 58749!

Notes: Kirk did not designate a single specimen as type of *C. fulvida var. linearis*, but gives "Dunedin, Aston" as locality and collector for the type. All of the specimens above were collected by B. C. Aston on the same day (02.02.1896) and probably from the same plant ("near Reservoir, Dunedin"). This can be deduced because Aston remarked on the labels of WELT 58747/A and WELT 58747/B: "I only know of one bush of this but one of our members says it is plentiful on the Silversteam Road". All of the specimens are from T. Kirk's herbarium with the exception of WELT 58665 (which is from the D. Petrie Herbarium ex B. C. Aston Herbarium) and were therefore available to Kirk when he described *C. fulvida var. linearis.* All of the specimens were labelled by Aston as *Cassinia fulvida* var. *rubra*. J. Fox (2000, unpubl. information) gives the following explanation:

> A note on WELT 58677a, a garden-grown specimen from Dunedin Waterworks collected in Jan. 1908 by H. J. Matthews in the D. Petrie Herbarium, states (in Petrie's hand) "Mr Kirk evidently changed the name of his var. *rubra* before publication". Presumably this was either to avoid confusion with Buchanan's earlier name of *Cassinia rubra* (Trans N.Z. Inst. 19 (1887): 216), or because he once thought that specimens he later named var. *linearis* were conspecific with Buchanan's *Cassinia rubra*. In the Students Fl. N.Z. (1899): 315, Kirk states that he is "indebted to Mr Buchanan for one of his specimens" (of *Cassinia rubra*). However no such specimen has been located amongst T. Kirk Herbarium collections of *Cassinia* at WELT. The distinctive pink apices of the phyllaries and the "*vauvilliersii*" type leaves of the Buchanan specimens would have clearly distinguished Kirk's "var. *linearis*" from Buchanan's "*rubra*".

Cassinia fulvida* var. *montana Allan, Flora N.Z. 1: 729 (1961).
TYPE COLLECTION: "V. D. Zotov, 15.01.1936, Arthur's Pass, Canterbury, Alt. 914 m".
HOLOTYPE: V. D. Zotov, CHR 9397!

***Cassinia leptophylla* var. γ** Hook.f., Flora N.Z. 1: 132 (1852)
≡ *Cassinia fulvida* Hook.f., Handbook N.Z. flora: 145 (1864).
TYPE COLLECTION: "Lyall" (on herbarium sheet); "Canterbury?, Lyall" (Hooker 1852).
HOLOTYPE: Lyall, Herbarium Hookerianum held at Kew (photograph seen), flowering piece on top right-hand corner of sheet.

Notes: Hooker (1852) indicated a specimen collected by Lyall to be his nomenclatural type. This specimen with "Lyall" written in Hooker's handwriting underneath can be found on one of Hookers herbarium sheets, held at Kew together with five specimens collected by Hector & Buchanan in Otago in 1863, and by an unknown collector at Hurunui Haven in 1864.

Cassinia retorta A. Cunn. ex DC., Prodromus 6: 154 (1838).
TYPE COLLECTION: "Hokianga river, 64; A. Cunningham"
HOLOTYPE: Allan Cunningham's New Zealand Herb. sheet held at Kew (photograph seen), fruiting piece on right-hand side of sheet, labelled 64 with a tag; label below gives the number 447.

Notes: The sheet which contains Cunningham's holotype of *Cassinia retorta* includes two additional specimens. Both of these are *C. leptophylla* collected from different localities (No. 49, ?-Bay Waikato inlet (9/64); No. 8?, Port Hills Nelson (8/64). A tag with the number 64 is attached to the holotype of *C. retorta*. A label below this specimen, clearly belonging to it as indicated by the number 64 noted on it, contains the number "No. 447", "*Cassinia leptophylla* ? R.Br." in brackets and the name *Cassinia retorta* with the reference to Cunningham's protologue. Two different names, presumably the collectors, and dates are given: A. Cunningham 1826 and R. Cunningham 1834 on this label. According to a label attached on top of this first label is the locality the Hokianga River

and the collector R. Cunningham in 1834. This specimen is regarded as a holotype, despite variance in collecting date and collector provided by two different labels on the sheet. No other material of *C. retorta* collected by A. or R. Cunningham could be found.

Cassinia rubra Buchanan, Trans. & Proc. N.Z. Inst. 19: 216 (1887)
≡ *Cassinia vauvilliersii* var. *rubra* (Buchanan) Cheeseman, Manual N.Z. flora: 346 (1906).
No type material of *C. rubra* could be located.

Cassinia spathulata Colenso, Trans. & Proc. N.Z. Inst. 22: 472 (1890)
≡ *Cassinia leptophylla* var. *spathulata* (Colenso) Kirk, Students' Flora: 315 (1899).
TYPE COLLECTION: "Hawkes Bay" on sheet, "Dry hills near the sea, Napier, Hawkes Bay" (Colenso 1890).
HOLOTYPE: W. Colenso Herbarium, WELT 24140!
ISOTYPE: Kirk Herbarium, WELT 58873!

Notes: Allan (1961: 727) indicated that the type is held at WELT, but did not specify a particular sheet. A label in the hand of T. F. Cheeseman, additional to that by Colenso is attached to WELT 24140. It reads: "*Cassinia leptophylla* Br. Hawkes Bay. Type of *C. spathulata*, Col."

Cassinia vauvilliersii* var. *albida Kirk, Students' Flora: 315 (1899)
≡ *Cassinia albida* (Kirk) Cockayne, Trans. & Proc. N.Z. Inst. 38: 374 (1906).
TYPE COLLECTION: "T. Kirk, Mount Fyffe, Kaikouras, Dec 14. 1889"
LECTOTYPE: T. Kirk Herbarium, WELT 58218! (here designated *fide* J. Fox)
ISOLECTOTYPE: T. Kirk Herbarium, WELT 29634!
LECTOPARATYPES: Kirk Herbarium, AK 30904!; Herbarium T. F. Cheeseman, AK 10304!; WELT 58314!, two upper pieces on sheet.

Notes: WELT 58218,WELT 29634, AK 30904 and AK 10304 were collected by T. Kirk from Mt Fyffe in the Seaward Kaikoura Range. WELT 58218 is dated "Dec 14. 1889", and

"albida" was underlined in red ink by Kirk. According to J. Fox (2000, unpubl. information) "a practice he seems to have applied to specimens either figured in publications or deemed to be 'type' material".

WELT 58314 is a mixed collection from Kirk's Herbarium and labelled by him. J. Fox (2000, unpubl. information) identified the two upper pieces as the specimen collected by John Buchanan at West Cape and listed in the original description of *Cassinia vauvilliersii* var. *albida* (Kirk 1899). They can be referred to a label in Kirk's hand that reads: "*Cassinia vauvilliersii*, Hook.f. var. *albida*. West Cape. Col[onial] Mus[eum]." A second label referring to the two lower pieces on the sheet gives "Kaikouras" and J. Rutland as collector. Since there has been no further material of *Cassinia vauvilliersii* var. *albida* recorded from "West Cape" it might be assumed that this type locality got mistaken.

Cassinia vauvilliersii* var. *pallida Allan, Flora N.Z. 1: 728 (1961).
TYPE COLLECTION: "H. H. Allan, 21.03.1951, Robinson Creek, Upper Awatere"
HOLOTYPE: CHR 87404!

Cassinia vauvilliersii* var. *serpentina Cockayne et Allan, Trans. & Proc. N.Z. Inst. 56: 22 (1926).
TYPE COLLECTION: "L. Cockayne, Mineral Belt Dun Mountain", Dec. 1916.
HOLOTYPE: L. Cockayne Herbarium: 1930, AK 31977!

Notes: AK 31977 is labelled as "syntype" in the collection of the Auckland War Memorial Museum. In their publication of *C. vauvilliersii* var. *serpentina* Cockayne and Allan (1926) clearly indicate as the type a specimen collected by L. Cockayne on the Mineral Belt of the Dun Mountain. Allan (1961: 728) refers to a specimen from the L. Cockayne Herbarium held in AK as the type. Since there is no other specimen of *C. vauvilliersii* var. *serpentina* known from the L. Cockayne Herbarium, AK 31977 appears to be the holotype for *C. vauvilliersii* var. *serpentina*.

Olearia xanthophylla Colenso, Trans. & Proc. N.Z. Inst. 20: 193 (1888).

TYPE COLLECTION: H. Hill, the High Plains, Waimarino, on the west side of Mount Tongariro, 1887.

HOLOTYPE: W. Colenso Herbarium, WELT 24144!

Ozothamnus vauvilliersii Hombr. et Jacq. ex Decne in Voy. Pôle Sud pl. 5 (1843).

≡ *Cassinia vauvilliersii* (Hombr. et Jacq.) Hook.f., Flora Antarctica 1: 29 (1847).

TYPE COLLECTION: "Iles Auckland-Sud de la Nouvelle Zelande, Hombron."

HOLOTYPE: M. Hombron 1841, P 179961 (photograph seen).

Notes: P 179961 includes five pieces of *Cassinia vauvilliersii* collected by Hombron. The piece in the bottom left-hand corner of the herbarium sheet remarkably resembles Plate 5 in the first issue of the folio atlas by Hombron & Jacquinot (1843). This illustration represents the first valid publication of *C. vauvilliersii* (as *Ozothamnus vauvilliersii*).

Table 5.1 '*Cassinia*' types.

Allan 1961	Herbarium	Type	Locality	Collector
C. amoena Cheeseman	**AK 10298** (*Herbarium T. F. Cheeseman*)	Lectotype	Cliffs from Kerr Point to North Cape	T. F. Cheeseman
	AK 10299 (*Herbarium T. F. Cheeseman*)	Isolectotype	Cliffs from Kerr Point to North Cape	T. F. Cheeseman
	WELT 58304 (Herbarium T. Kirk ex Herb. T. F. Cheeseman)	Isolectotype	Cliffs from Kerr Point to North Cape	T. F. Cheeseman
C. fulvida Hook. f.	**K** (*Herbarium Hookerianum 1867*)	Holotype	"Canterbury" (Hook. f. loc. cit.)	Lyall
C. fulvida var. linearis Kirk	**WELT 58747/A** (*T. Kirk Private Herbarium*)	Lectotype	near Reservoir, Dunedin	B. C. Aston
	WELT 58665 (*D. Petrie Herbarium*)	Isolectotype	near Reservoir, Dunedin	B. C. Aston
	WELT 58747/B (*T. Kirk Private Herbarium*)	Isolectotype	near Reservoir, Dunedin	B. C. Aston
	WELT 58748/A (*T. Kirk Private Herbarium*)	Isolectotype	near Reservoir, Dunedin	B. C. Aston
	WELT 58748/B (*T. Kirk Private Herbarium*)	Isolectotype	near Reservoir, Dunedin	B. C. Aston
	WELT 58749 (*T. Kirk Private Herbarium*)	Isolectotype	near Reservoir, Dunedin	B. C. Aston
C. fulvida var. *montana* Allan	**CHR 9397**	Holotype	Arthur's Pass, Canterbury,	V. D. Zotov
C. leptophylla (G.Forst.) R.Br.	**K** (*The Forster Herbarium. Presented by the Corporation of Liverpool, August, 1885*)	Lectotype	Queen Charlotte Sound?	G. Forster?
C. leptophylla var. *spathulata* (Colenso) Kirk	**WELT 24140** (*W. Colenso Herbarium*)	Holotype	Dry hills near the sea, Napier	W. Colenso
	WELT 58873 (*T. Kirk Herbarium*)	Isotype	Dry hills near the sea, Napier	W. Colenso
C. retorta A. Cunn. *ex DC.*	**K** (*Allan Cunningham's New Zealand Herbarium. Presented by Robert Heward Esq. 1862*)	Holotype	Hokianga River	A. Cunningham
C. vauvilliersii (Hombr. et Jacq.) Hook.f.	**P 179961**	Holotype	Auckland Is.	J. B. Hombron
C. vauvilliersii var. *albida* Kirk	**WELT 58218** (*T. Kirk Herbarium*)	Lectotype	Mt. Fyffe	T. Kirk
	WELT 29634 (*T. Kirk Herbarium*)	Isolectotype	Mt. Fyffe	T. Kirk
	WELT 58314 (*Colonial Museum*)	Lectoparatype	West Cape	J. Buchanan?
	AK 10304 (*Herbarium T. F. Cheeseman*)	Lectoparatype	Kaikoura Mountains	T. Kirk
	AK 30904 (*Herbarium T. Kirk*)	Lectoparatype	Kaikoura Mountains	T. Kirk
C. vauvilliersii var. *canescens* (Cockayne) Allan	**WELT 58358** (*L. Cockayne Herbarium No.9093*)	Holotype	Mt. Fyffe	L. Cockayne
C. vauvilliersii var. *pallida* Allan	**CHR 87404**	Holotype	Robinson Creek, Upper Awatere	H. H. Allan
C. vauvilliersii var. *serpentina* Cockayne et Allan	**AK 31977** (*Herbarium Leonard Cockayne: 1930*)	Holotype	Nelson, Dun Mountains	L. Cockayne

Taxonomic treatment

The formal taxonomic treatment that follows is prepared for publication and repeats much of the material in previous chapters. This thesis is not intended by the author to be place of publication of names proposed in it.

Ozothamnus leptophyllus (G.Forst.) Breitw. et J.M. Ward, N.Z.J.Bot. 35: 125-128 (1997)
≡ *Calea leptophylla* G.Forst., Prodromus: 55 (1786).
≡ *Cassinia leptophylla* (G.Forst.) R.Br., Trans. Linn. Soc. Lond. 12: 126 (1817).
Lectotype: Queen Charlotte's Sound?, G. Forster, K (photograph seen), fruiting piece on left side of sheet.

Much-branched shrub, (0.5-)0.8-2(-3) m tall. Branchlets tomentose. Leaves persistent, coriaceous, adaxially almost glabrous to densely hairy, abaxially densely tomentose. Branchlet and leaf tomentum white, often overlain with yellowish exudate from small glandular hairs. Lamina oblong or narrow-obovate or elliptic, 2-12(-18) × 1-4(-5) mm; apex acute, obtuse or rounded, rarely retuse; base decurrent; margin entire, more or less revolute; petiole very short, erect, closely appressed to branch. Capitula homogamous, 3.5-7(-9) × 1-4(-5) mm, numerous in terminal, more or less dense corymbs. Corymbs aggregated into rounded, dense or diffuse panicles. Involucre campanulate or turbinate, of many or few short, free, partly overlapping bracts arranged in a compressed spiral. Outer involucral bracts ovate or oblong, sparsely to densely hairy, concave, usually greenish brown, sometimes reddish towards the apex; edges tawny, scarious; margins entire or torn; tips tawny, scarious, or tawny with a flat white or cream, erose or torn apex. Inner involucral bracts 3-5 × 0.4-1 mm, oblong to linear-oblong but widening towards the tip, slightly concave at the base; margins scarious, entire or torn-ciliate; tips white, more or less radiating, of slightly variable size and shape, margins entire or sometimes erose. Receptacle convex, narrow, usually with receptacle scales, with white, erect or rarely radiating tips, slightly exceeding corollas. Florets 4-15(-25) per capitulum, perfect, tubular, 5-toothed. Corolla tube cylindrical, slightly expanded at base, lobes spreading to reflexed, glandular. Anthers sagittate at the base, produced into slender tails. Style swollen or club-shaped at the base, style-branches terete, apex expanded, truncate, papillose, with dorsal cells immediately behind apex projecting slightly. Achenes 0.6-1.6 mm long, slightly

compressed, slightly narrowed to base, usually with glandular hairs, sometimes with scattered antrorse twin hairs. Pappus-hairs in a single series, 2-4 mm long, slender, scarcely to distinctly thickened at apex, barbellate, connate at the base.

Key to the subspecies of *Ozothamnus leptophyllus*

1 Receptacle with few or no scales among the florets *2*

Receptacle usually with numerous scales among the florets *3*

2 Leaves 6-13(-18) × 2.5-4.5 mm, abaxial surface white, very rarely pale yellow, clothed with extremely dense tomentum ..... **subsp. *amoenus***

Leaves 3.5-6 × 1(-2) mm, abaxial surface yellowish to orange yellow, tomentum on abaxial surface moderately dense ..... **subsp. *fulvidus***

3 Leaves 2-5 × 1-2.5 mm; coastal *4*

Leaves 3.5-13 × (1.8-)2.5-4 mm; not coastal *5*

4 Capitula 5-6 × 2-3 mm, in large compound panicles; florets c. (5-)7-14 per capitulum ..... **subsp. *leptophyllus***

Capitula 7-7.5(-9) × 3-4.5(-5) mm, usually in small simple panicles; florets 12-18(-20) per capitulum ..... **subsp. *retortus***

5 Leaves mostly acute, abaxial surface white, very rarely pale yellow; tomentum on branchlets and abaxial surface of leaves very dense; young leaves extremely glutinous; midvein moderately to strongly evident on abaxial surface of leaves; outer involucral bracts never reddish, bright red, or pink towards the tips ..... **subsp. *albidus***

Leaves usually obtuse or rounded, rarely acute or retuse, abaxial surface pale yellowish green to deep orange-yellow; tomentum on branchlets and abaxial surface of leaves moderately dense; young leaves not or slightly glutinous; midvein slightly to moderately evident on abaxial surface of leaves; outer involucral bracts often reddish, bright red, or pink towards the tips ..... **subsp. *vauvilliersii***

Table 5.2 Some distinguishing features of the subspecies of *Ozothamnus leptophyllus*.

	subsp. *leptophyllus*	subsp. *albidus*	subsp. *amoenus*	subsp. *fulvidus*	subsp. *retortus*	subsp. *vauvilliersii*
Growth habit	straggling shrub	slightly straggling, round-topped shrub	erect, round-topped shrub	straggling shrub	slightly straggling shrub	erect, round-topped shrub
Height	100-150(-200) cm	80-100 cm	up to 100 cm	80-150(-300) cm	80-150 cm	70-200 cm
Main branches	mostly erect, 2-3	somewhat spreading, >5	somewhat spreading, >5	mostly erect, 2-3	inner main branches erect, outer branches sometimes decumbent or slightly prostrate, >5	somewhat spreading, outer branches sometimes decumbent or prostrate, >5
Branchlets	c. 12 per 5 cm branch at angle of c. 27°, upright, more or less fastigiate, sometimes descending or deflexed, 1.3-2 mm diameter. new branchlets developing below young terminal inflorescence while the flowers are still in bud.	c. 5 per 5 cm branch at angle of 47°, slightly spreading, never descending or deflexed, c. 1.6 mm diameter. new branchlets developing below old terminal inflorescence after fruiting.	c. 8 per 5 cm branch at angle of 53°, slightly spreading, never descending or deflexed, 1.5-2 mm diameter. new branchlets developing below old terminal inflorescence after fruiting.	c. 11 per 5 cm branch at angle of c. 34°, more or less fastigiate, sometimes descending or deflexed, c. 1.4 mm diameter. new branchlets developing below young terminal inflorescence while the flowers are still in bud.	c. 15 per 5 cm branch at angle of c. 37°, sometimes descending or deflexed, c. 0.7-1.5(-2) mm diameter. new branchlets developing below old terminal inflorescence after fruiting.	c. 7 per 5 cm branch at angle of c. 42°, never descending or deflexed, c. 1.6 mm diameter. new branchlets developing below old terminal inflorescence after fruiting.
Leaf posture	lamina of young leaves erect (30-40°), later spreading (70°-90°)	lamina of young leaves erect (c. 30°), later spreading (64-90°) sometimes recurved	c. 46-49°	lamina of young leaves erect (c. 30°), later spreading (64-90°)	>90°, sometimes strongly recurved	lamina of young leaves erect (c. 30°), later spreading (60°) sometimes recurved
Leaf lamina	narrow-oblong to oblong, 2-5 × 1.3-2(-2.5) mm	narrow-obovate or elliptic to narrow-elliptic, 6-13 × 2.5-4 mm	narrow-obovate, 6-13(-18) × 2.5-4.5 mm	narrow-oblong to narrow-obovate, sometimes narrow elliptic, 3.5-6 × 1(-2) mm	narrow-obovate, sometimes oblong, (2-)3-4(-5) × (1-)1.5-2 mm	narrow-obovate or elliptic to narrow-elliptic, 4.5-10 × 2.5-4 mm
Leaf apex	obtuse to acute, sometimes rounded	acute	mostly rounded, sometimes obtuse	obtuse to acute, sometimes rounded	rounded to obtuse	obtuse or rounded, rarely acute or retuse
Leaf tomentum						
adaxially	sparsely to moderately covered with fine hairs	densely covered with fine hairs	thinly covered with fine hairs	glabrous or rarely sparsely covered with fine hairs	sparsely to densely covered with fine hairs	sparsely to densely covered with fine hairs
abaxially	very dense	very dense	extremely dense	moderately dense	extremely dense	moderately dense to dense
Leaf and branchlet exudates	not or very sparsely overlain with greenish to pale yellow exudates	sometimes very sparsely overlain with pale yellow-green exudates	not, or very sparsely overlain with pale yellow-green exudates	always densely overlain with bright yellow to deep orange-yellow exudates	not, or sometimes sparsely to densely overlain with yellow exudates	always overlain with pale yellow-green or bright yellow to orange-yellow exudates of varying density
Leaf margin	strongly to very strongly recurved	moderately recurved	strongly recurved	slightly to very strongly recurved	strongly recurved	slightly to moderately recurved
Leaf midvein on abaxial surface	strongly to very strongly evident	moderately to strongly evident	strongly evident	slightly to very strongly evident	very strongly evident	midvein slightly to moderately evident

Table 5.2 continued

	subsp. *leptophyllus*	subsp. *albidus*	subsp. *amoenus*	subsp. *fulvidus*	subsp. *retortus*	subsp. *vauvilliersii*
Panicles	mostly diffuse, often several times compound, flat or sometimes slightly rounded, 23-60 mm diameter	dense to slightly diffuse, sometimes several times compound, rounded, 30-60 mm diameter	dense, rounded, 13 mm diameter	diffuse to dense, often several times compound, flat or sometimes slightly rounded, 12-60 mm diameter	slightly diffuse, often simple, rounded, 15(-35) mm diameter	dense to diffuse, sometimes several times compound, rounded, 15-45 mm diameter
Capitula/panicle	50-100(-250)	35-185	9-12(-17)	17-40(-300)	6-11	14-50(-100)
Capitulum size	5-6 × 2-3 mm	5.5-6 × 4 mm	5.5-6.5 × 2.5-3 mm	(3.5-)4-6 × (1-)1.5-3 mm	7-7.5(-9) × 3-4.5(-5) mm	5-6.5 × 2.5-4 mm
Outer involucral bract number	11-16	10	9-11	8	16-20	9-14
Outer involucral bract colour	greenish brown	greenish brown or tawny, with a small cream scarious erose apex	greenish brown to tawny with a small cream scarious erose apex	greenish brown with dense yellow exudates	tawny green, yellowish towards the tips	greenish brown; often reddish, bright red, or pink towards the tips, sometimes with a small cream scarious erose apex
Inner involucral bract number	7	9-16	7-9	7	7(-10)	9-15
Inner involucral bract size	3.6-4 × 0.7-0.8 mm	4 × 1 mm	4.4 × 0.7 mm	(3-)3.5 × (0.4-)0.5-1 mm	5 × 1 mm	4-4.5 × 0.7-1 mm
Receptacle scale number	(3-)5-11(-15)	10-16	0	(0)1-5	20-27	5-17
Florets/capitulum	(5-)7-14	17	4-6	3-9	12-18(-20)	9-20
Corolla tube	2.5 mm long	3 mm long	3 mm long	2.4 mm long	3.5-4 mm long	2-3 mm long
Corolla lobes	0.7 × 0.3 mm	0.6 × 0.3 mm	0.6 × 0.3 mm	0.55 × 0.3 mm	0.8 × 0.45 mm	0.4-0.7 × 0.3-0.4 mm
Style	2-2.3 mm long	2.5 mm long	2.5 mm long	1.7-2.5 mm long	3 mm long	1.8-2.8 mm long
Style branches	0.7-0.9 mm	0.6-0.9 mm	0.8 mm	0.5-0.9 mm	1 mm	0.5-0.8 mm
Achenes	0.8-1 × 0.3-0.4 mm	1.6 × 0.6 mm	1.2 × 0.6 mm	0.8-1 × 0.3-0.5 mm	1-1.5 × 0.5 mm	0.6-1(-1.6) × 0.3-0.6 mm
Achenial twin hairs	absent or very sparse	very variable (absent to dense)	very dense	very variable (absent to dense)	absent or sparse	absent to dense
Achenial glandular hairs	absent or very sparse	absent or sparse	very dense	very variable (absent to dense)	absent or sparse	absent or sparse
Pappus length	2.5-3 mm long	3.5 mm long	up to 3.5 mm long	(2-)2.4-3.5 mm long	up to 4 mm long	3-3.5 mm long

Ozothamnus leptophyllus* subsp. *leptophyllus

= *Cassinia spathulata* Colenso, Trans. & Proc. N.Z. Inst. 22: 472 (1890).

≡ *Cassinia leptophylla* var. *spathulata* (Colenso) Kirk, Students' Flora: 315 (1899).

Holotype: Hawkes Bay, W. Colenso, WELT 24140!; isotype WELT 58873!

DESCRIPTION: Straggling shrub, 100-150(-200) cm tall, main branches mostly erect, 2-3. Branchlets c. 12 per 5 cm branch at angle of c. 27°, sometimes descending or deflexed, 1.3-2 mm diameter, clothed with dense to very dense tomentum, not or very sparsely overlain with greenish to pale yellow exudates, new branchlets developing below young terminal inflorescence while the flowers are still in bud. Leaves c. 9 per cm branchlet; orientation of young leaves 30-40°, mature leaves 70-90°; lamina narrow-oblong to oblong, sometimes narrow-obovate, 2-5 × 1.3-2(-2.5) mm; apex obtuse to acute, sometimes rounded; adaxial surface of young and mature leaves sparsely to moderately covered with fine hairs; abaxial surface clothed with very dense white tomentum, not or very sparsely overlain with greenish to pale yellow exudates; margins strongly to very strongly recurved; midvein strongly to very strongly evident on abaxial surface. Panicles mostly diffuse, often several times compound, flat or sometimes slightly rounded, 23-60 mm diameter. Pedicels 1.7-2.7 mm long. Capitula 50-100 in less compound panicles, up to 250 in the large, compound panicles, 5-6 × 2-3 mm. Outer involucral bracts c. 11-16, narrow-ovate, greenish brown; tips acute, not spreading; abaxial surface slightly to moderately hairy. Inner involucral bracts c. 7, 3.6-4 × 0.7-0.8 mm, pale green below; tips white, obtuse, more or less radiating; abaxially glabrous or sometimes with sparse hairs. Receptacle scales (3-)5-11(-15), sometimes more, with erect white tips. Florets c. (5-)7-14 per capitulum. Corolla tube c. 2.5 mm long, lobes 0.7 × 0.3 mm. Style c. 2-2.3 mm long; style branches 0.7-0.9 mm. Achenes 0.8-1 × 0.3-0.4 mm; twin hairs and glandular hairs almost absent, only sometimes present and very sparse. Pappus hairs 2.5-3 mm long, moderately thickened at tips; apical cells c. 4; barbellae short, hardly spreading. Chromosome number: not known. FL Jan-Apr, FT Feb-May.

DISTRIBUTION (Fig. 5.2 A): Distributed in central portion of New Zealand. From latitude 37°30'S extending southwards coastally to latitude 40°30'S on the west coast of the South Island and to latitude 42°S on the east coast of the South Island. Predominantly coastal, extending inland at the Kaikoura Ranges.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *leptophyllus* is recognised by its slender and upright habit, branchlets and leaves clothed in dense white tomentum, and capitula in very large, spreading panicles. It is distinguished from *O. leptophyllus* subsp. *fulvidus* by a denser tomentum that is not or far less overlain with yellow exudates, and more florets and receptacle scales per capitulum. Long, slender and fastigiate side shoots which exceed the large panicles while they are still in flower and smaller narrower leaves distinguish *O. leptophyllus* subsp. *leptophyllus* from *O. leptophyllus* subsp. *vauvilliersii*, *O. leptophyllus* subsp. *albidus*, and *O. leptophyllus* subsp. *amoenus*. Although resembling *O. leptophyllus* subsp. *retortus* in some of the vegetative characters, it is clearly distinguished from this subspecies by smaller capitula arranged in larger panicles.

VARIATION: Variation occurs in colour and density of exudates. On sand dunes the growth form can be modified to low, more or less prostrate shrubs, as seen in the northern range of the distribution of *O. leptophyllus* subsp. *leptophyllus*.

REPRESENTATIVE SPECIMENS: SOUTH ISLAND: CANU 38581, Queen Charlotte Drive, Grove Arm, Marlborough, I. Schönberger 139B; CHR 188752, Stephens Island, Cook Strait, Marlborough, B. H. Macmillan 68/13815; WELT 58892, Queen Charlotte Sound, Grove Reach [Grove Arm], Marlborough, W. R. B. Oliver; WELTU 19505, Cape Farewell, Nelson, P. Garnock-Jones 2343; NORTH ISLAND: AK 233648, Potikirua Road, west of Lottin Point, Gisborne, H. K. Taylor; CANU 38572, Makorori Beach, Gisborne, T. Jenkins (I. Schönberger 58B); CANU 38576, Te Korohiwa Rocks, Titahi Bay, Wellington, I. Breitwieser & R. W. Vogt 2072; CHR 108651, Titahi Bay, Wellington, H. H. Allan ; CHR 21936, Cooks Cove, Tolaga Bay, Gisborne, V. D. Zotov; CHR 226768, Near Lighthouse, East Cape, Gisborne, W. R. Sykes 162/72; OTA 23159, Akatarawa Valley, Tararua Range, Wellington, A. F. Mark; WELTU 16015, Rimutaka State Forest, Breitwieser 1024 & R. W. Vogt.

CONSERVATION STATUS: Not threatened.

ETYMOLOGY: The epithet *leptophyllus* refers to the "slender" leaves of this subspecies.

ILLUSTRATIONS: Banks' Florilegium (Banks et al. 1980-1990), Plate 484 (Part XXIII New Zealand); Wilson (1982, p. 63: 39); Wilson & Galloway (1993, p. 207: 108); Poole & Adams (1994, p. 203); Salmon (1991, p. 15: 53).

Ozothamnus leptophyllus **subsp.** ***albidus*** (Kirk) Schönberger comb. et stat. nov.
≡ *Cassinia vauvilliersii* var. *albida* Kirk, Students' Flora: 315 (1899).
≡ *Cassinia albida* (Kirk) Cockayne, Trans. & Proc. N.Z. Inst. 38: 374 (1906).
Lectotype: Mt Fyffe, Kaikouras; T. Kirk, 14.12.1889, T. Kirk Herbarium, WELT 58218!; isolectotype: WELT 29634!
= *Cassinia albida* var. *canescens* Cockayne, Trans. & Proc. N.Z. Inst. 38: 369 (1906).
≡ *Cassinia vauvilliersii* var. *canescens* (Cockayne) Allan, Flora N.Z. 1: 728 (1961).
Holotype: Mt Fyffe, L. Cockayne, 07.10.1905, L. Cockayne Herbarium No. 9093, WELT 58358!

DESCRIPTION: Slightly straggling, round-topped shrub 80-100 cm tall, main branches somewhat spreading, >5. Branchlets c. 5 per 5 cm branch at angle of c. 47°, never descending or deflexed, c. 1.6 mm diameter, clothed with very dense tomentum, not or very sparsely overlain with pale yellow-green exudates, new branchlets developing below old terminal inflorescence after fruiting. Leaves c. 6 per cm branchlet; orientation of young leaves c. 30°, mature leaves 64-90° sometimes recurved; lamina narrow-obovate or elliptic to narrow-elliptic, 6-13 × 2.5-4 mm, apex acute; adaxial surface of young and mature leaves densely covered with fine hairs; abaxial surface clothed with very dense white tomentum, sometimes very sparsely overlain with pale yellow-green exudates; young leaves extremely glutinous; leaves remain more or less glutinous throughout the season; margins moderately recurved; midvein moderately to strongly evident on abaxial surface. Panicles dense to slightly diffuse, sometimes several times compound, rounded, 30-60 mm diameter. Pedicels 2-6 mm long. Capitula 35-185 per panicle, c. 5.5-6 × 4 mm. Outer involucral bracts c. 10, broad-ovate, greenish brown or tawny with a small cream scarious erose tips; tips not or very slightly spreading; abaxial surface densely hairy. Inner involucral bracts 9-16, 4 × 1 mm, tawny to pale green with white radiating tips, abaxially moderately hairy. Receptacle scales 10-16, with erect white tips. Florets c. 17 per

capitulum. Corolla tube c. 3 mm long, lobes c. 0.6 × 0.3 mm. Style c. 2.5 mm long; style branches 0.6-0.9 mm. Achenes 1.6 × 0.6 mm; density of twin hairs very variable; glandular hairs absent or sparse. Pappus hairs c. 3.5 mm long, slightly thickened at tips; apical cells 4-5; barbellae short, hardly spreading. Chromosome number: $2n = 26$-28. FL Jan-Apr, FT Dec-May.

DISTRIBUTION (Fig. 5.2 B): Confined to the Kaikoura Mountains and their vicinity, along the seaward face, extending in a westerly direction as far as the Clarence River and the middle portion of the Wairau Valley. Subalpine scrub, rarely lowland river-flats.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *albidus* is recognised by very large, narrow-obovate leaves with acute apices and a very dense white tomentum, not or very sparsely overlain with pale yellow-green exudates abaxially, and large capitula with many (c. 17) florets arranged in large, slightly diffuse panicles. Leaves and branchlets are glutinous. The ranges for the exudate and tomentum density for the *O. leptophyllus* subsp. *albidus*, *O. leptophyllus* subsp. *leptophyllus* and *O. leptophyllus* subsp. *amoenus* are very similar, but distinguish *O. leptophyllus* subsp. *albidus* from *O. leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii* and var. *collinus* which have a less dense tomentum overlain by bright yellow exudates. Thick pappus hairs that are not much spreading at the tips, and short basal appendages of the anthers distinguish *O. leptophyllus* subsp. *albidus* from all the varieties of *O. leptophyllus* subsp. *vauvilliersii*.

VARIATION: Variation occurs in the density of the tomentum on the adaxial surface of the leaves. Some plants seem to lose their tomentum on the adaxial surface of the leaves or an increased amount of leaf surface waxes makes the presence of the hairs less obvious in older leaves. In other plants, the tomentum on the adaxial surface of the leaves remains obvious, giving the plants a pale green appearance.

REPRESENTATIVE SPECIMENS: CANU 38569, Mt Fyffe, Marlborough, I. Schönberger 33B; CHR 117180, Near Kaikoura, Mount Fyffe, Marlborough, P. Wardle; CHR 151069, Mount Baldy, Marlborough, H. Jenkins; CHR 166805, North Branch, Hapuku River, near Kaikoura, Marlborough, P. Wardle; CHR 218442, South ridge of Mount Fyffe, in subalpine scrub on steep, west-facing slopes Marlborough, D. R. Given 71109; CHR

333837, Carse Herbarium no, 1587/3, Ure River, Benmore, Marlborough, A. Wall; WELT 58283, Wardle Bush, Nelson, L. Cockayne.

CONSERVATION STATUS: Not threatened.

ETYMOLOGY: The epithet *albidus* refers to the dense tomentum on leaves and branchlets giving this subspecies a "somewhat white" or "whitish" appearance.

Ozothamnus leptophyllus* subsp. *amoenus (Cheeseman) Schönberger comb. et stat. nov. ≡ *Cassinia amoena* Cheeseman, Trans. & Proc. N.Z. Inst. 29: 391 (1897).
Lectotype: North Cape, T. F. Cheeseman, Herbarium T. F. Cheeseman, Jan. 1896, AK 10298!; isolectotypes: AK 10299! WELT 58304!

DESCRIPTION: Erect, round-topped shrub to 1 m tall, main branches somewhat spreading, >5. Upper branchlets c. 8 per 5 cm branch at angle of 53°, never descending or deflexed, 1.5-2 mm diameter, clothed with very dense white tomentum, not or very sparsely overlain with yellow-green exudates, new branchlets developing below old terminal inflorescence after fruiting. Leaves c. 6-8 per cm branchlet; lamina orientation c. 46-49°, lamina narrow-obovate, 6-13(-18) × 2.5-4.5 mm, apex mostly rounded, sometimes obtuse; adaxial surface of young and mature leaves thinly covered with fine, white hairs; abaxial surface clothed with extremely dense white tomentum, not or very sparsely overlain with pale yellow-green exudates; margins strongly recurved; midvein strongly evident on abaxial surface. Panicles dense, rounded, c. 13 mm diameter. Pedicels 0.5-2 mm long. Capitula 9-12(-17) per panicle, 5.5-6.5 × 2.5-3 mm. Outer involucral bracts c. 9-11, ovate, greenish brown to tawny with small cream scarious erose erect tips; abaxial surface densely hairy. Inner involucral bracts c. 7-9, 4.4 × 0.7 mm, tawny to greenish brown with short white radiating tips, abaxially moderately hairy. Receptacle scales absent. Florets 4-6 per capitulum. Corolla tube c. 3 mm long, lobes c. 0.6 × 0.3 mm. Style c. 2.5 mm long; style branches c. 0.8 mm. Achenes 1.2 × 0.6 mm; twin hairs and glandular hairs very dense. Pappus hairs up to 3.5 mm long, moderately thickened at tips; apical cells up to 6; barbellae long, spreading. Chromosome number: $2n$ = 26-28. FL (Dec)Jan-Mar, FT Feb-Apr.

DISTRIBUTION (Fig. 5.2 C): Known only from the North Cape area between Surville Cliffs and Kerr Point. Coastal ultramafic rock outcrops and clay soils in vicinity of coastal cliffs.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *amoenus* is recognised by its small size, the absence of receptacle scales, large narrow-obovate leaves with mostly rounded apices, an extremely dense tomentum on the abaxial surface of the leaves, and densely arranged twin hairs and glandular hairs on the achenes. None of these character states is unique for this subspecies but the combination is unique. It is distinguished from *O. leptophyllus* subsp. *retortus*, *O. leptophyllus* subsp. *leptophyllus* and *O. leptophyllus* subsp. *fulvidus* by its habit, much smaller size, larger leaves, and broader heads with more numerous florets; from *O. leptophyllus* subsp. *albidus* and *O. leptophyllus* subsp. *vauvilliersii* by the absence of receptacle scales.

REPRESENTATIVE SPECIMENS: AK 24464, Kerr Point, North Auckland, R. C. Cooper, AK 24464; AK 31992, North Cape District, North Auckland, H. B. Auckland, H. B. Matthews; CANU 38583, Surville Cliffs, North Auckland, cultivated (I. Schönberger 185); CANU 9649, North Cape between N Cape and Kerr Point, North Auckland, I. A. Worley; CHR 174877, Kerr Point, North Auckland, L. J. Dumbleton; CHR 178178, 1.6 km south of Kerr Point, North Auckland, G. C. Kelly; CHR 333817, Hill side near North Cape, North Auckland, H. Carse 1588/2; CHR 354810, north-west of North Cape, top of Surville Cliffs, North Auckland, A. P. Druce; CHR 355755, North Cape near serpentine quarry, North Auckland, D. R. Given 11555 and J. Bartlett; CHR 355755, North Cape near serpentine quarry, North Auckland, D. R. Given 11558 and J. Bartlett; CHR 475135, Surville Cliffs near Ngawhenua Stream, North Auckland, P. J. de Lange 1249 and G. M. Crowcroft; CHR 518396, Surville Cliffs, North Cape, North Auckland, M. Dawson G382/96; OTA 2439, North Cape, North Auckland, G. T. S. Baylis; WELT 58302, North Cape, North Auckland, T. F. Cheeseman; WELT 58308, North Cape Peninsula, North Auckland, W. R. B. Oliver.

CONSERVATION STATUS: Although restricted to a small geographical area at the North Cape, this subspecies comprises several moderate-size populations. It is not threatened.

ETYMOLOGY: The epithet *amoenus* was given by Cheeseman because he regarded this plant as "beautiful" or "pleasant".

ILLUSTRATIONS: Cheeseman (1914, Plate 107); Salmon (1991, p. 66: 264); Poole & Adams (1994, p. 203); Eagle (1986, Vol. II: 262).

Ozothamnus leptophyllus* subsp. *fulvidus (Hook.f.) Schönberger comb. et stat. nov.
≡ *Cassinia leptophylla* var. γ Hook.f., Flora N.Z. 1: 132 (1852) pro parte.
≡ *Cassinia fulvida* Hook.f., Handbook N.Z. flora: 145 (1864) pro parte.
Holotype: Canterbury?, Lyall, K (photograph seen), flowering piece on right-hand side top corner of sheet.
= *Cassinia fulvida var. linearis* Kirk, Students' Flora: 315 (1899).
Lectotype: Near Reservoir, Dunedin, B. C. Aston, 02.02.1896, T. Kirk Private Herbarium, WELT 58747/A!; isolectotypes: WELT 58747/B!, WELT 58748/A!, WELT 58665!, WELT 58748/B!, WELT 58749!.

DESCRIPTION: Straggling shrub, 80-150(-300) cm tall, main branches mostly erect, 2-3. Branchlets c. 11 per 5 cm branch at angle of c. 34°, sometimes descending or deflexed, c. 1.4 mm diameter, clothed with sparse to dense tomentum always overlain with bright yellow to deep orange-yellow exudates, new branchlets developing below young terminal inflorescence while the flowers are still in bud. Leaves c. 8 per cm branchlet; orientation of young leaves c. 30°, mature leaves 64°-90°; lamina narrow-oblong to narrow-obovate or sometimes narrow-elliptic, ± 3.5-6 × 1(-2) mm; apex obtuse to acute, sometimes rounded; adaxial surface of young and mature leaves usually glabrous, rarely sparsely covered with fine hairs; abaxial surface clothed with moderately dense tomentum, always densely overlain with bright yellow to deep orange-yellow exudates; margins slightly to very strongly recurved; midvein slightly to very strongly evident on abaxial surface. Panicles diffuse to dense, often several times compound, flat or sometimes slightly rounded, 12-60 mm diameter. Pedicels 0.6-2 mm long. Capitula 17-40 in less compound panicles, up to 300 in large, compound panicles, (3.5-)4-6 × (1-)1.5-3 mm. Outer involucral bracts c. 8, narrow-ovate, greenish brown with yellow exudates, sometimes reddish towards the tips; tips acute, not spreading; hairs on abaxial surface sparse. Inner involucral bracts c. 7,

(3-)3.5 × (0.4-)0.5-1 mm, tawny to pale green below; tips white, obtuse, more or less radiating; abaxially glabrous or sometimes sparsely hairy. Receptacle scales (0-)1-5, with erect white tips. Florets c. 3-9 per capitulum. Corolla tube c. 2.4 mm long, lobes 0.55 × 0.3 mm. Style 1.7-2.5 mm long; style branches 0.5-0.9 mm. Achenes 0.8-1 × 0.3-0.5 mm; twin hairs and glandular hairs absent to dense. Pappus hairs (2-)2.4-3.5 mm long, moderately thickened at tips; apical cells c. 4; barbellae very short, hardly spreading. Chromosome number: not known. FL Jan-Apr, FT Feb-May.

DISTRIBUTION (Fig. 5.2 D): Restricted to the South Island, from latitude 41°30'S to 46°S. Coastal and lowland, rarely montane shrubland and grassland.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *fulvidus* is recognised by its slender branchlets, small narrow leaves with bright yellow to orange exudates, and small capitula with only a few (3-9), small florets, arranged in large spreading panicles. These features distinguish *O. leptophyllus* subsp. *fulvidus* from *O. leptophyllus* subsp. *amoenus*, *O. leptophyllus* subsp. *albidus*, *O. leptophyllus* subsp. *retortus* and *O. leptophyllus* subsp. *vauvilliersii*. The main differences between *O. leptophyllus* subsp. *fulvidus* and *O. leptophyllus* subsp. *leptophyllus* are the striking colour differences in their leaf and branchlet tomentum due to differences in tomentum density and exudate density and colour (less dense and overlain by bright yellow exudates in *O. leptophyllus* subsp. *fulvidus*, white and more dense in *O. leptophyllus* subsp. *leptophyllus*), and the smaller number of florets and receptacle scales in the capitula of *O. leptophyllus* subsp. *fulvidus*.

VARIATION: Some plants have dark yellow to orange exudates on leaves and branchlets. This colour variation is not geographical and is retained in cultivation. Examples are CANU 38587 from a beach north of Christchurch, Canterbury, and CANU 38572 from rocky outcrops in the Clutha Valley, Otago. In Otago and South Canterbury, plants are occasionally found with a denser tomentum on the abaxial surface of slightly longer leaves (e.g. CANU 38570 from the Orari River Rd towards Mt Peel). The leaves are very narrow and have very strongly recurved margins. These plants have been described as *Cassinia fulvida var. linearis* Kirk. Allan (1961) did not accept this taxon and suggested that *Cassinia fulvida var. linearis* might be of hybrid origin.

REPRESENTATIVE SPECIMENS: CANU 38562, Blythe Rd between Napenape and Motunau Beach, Canterbury, I. Schönberger 7C; CANU 38563, Stonyhurst Rd between HW1 and Stonyhurst, Canterbury, I. Schönberger 8G; CANU 38570, Orari River Rd towards Mt Peel, Canterbury, I. Schönberger 34A; CANU 38572, Clutha Valley near Crippletown, Otago, I. Schönberger 43A; CANU 38586, North of Motunau near Greta Canyon, Canterbury, I. Schönberger 5A; CANU 38587, Between Spencer Park and Waimairi, Christchurch, Canterbury, I. Schönberger 77A; CHR 109004, Leithfield Beach, Canterbury, R. Mason 7107; CHR 127075, Puhi Puhi Stream, tributary of Hapuku River, Marlborough, E. Edgar; CHR 201506, Pleasant Point foreshore, Canterbury, B. P. Molloy; CHR 201560, Woodend Beach, Canterbury, B. P. Molloy; CHR 217276, Pareora Gorge, South of Timaru, South Canterbury, J. A. Langbein; CHR 228157, Between Fruitlands and Butchers Dam, near Alexandra, Otago, P. Wardle; CHR 333806, Carse Herbarium, no.1589/2, Dunedin, Otago, D. Petrie; CHR 33413, Happy Valley Station, North Canterbury, A. J. Healy; CHR 377142, Parikawa, Marlborough, G. Brownlie 518; CHR 417834, Bendigo Cromwell, Otago, P. N. Johnson 242; CHR 439126, Herbarium of Bernice P. Bishop Museum. no.3297. Acc. no. 1982.262, Spencer Park (Christchurch), Canterbury, C. H. Lamoureux; CHR 494035, Pigeon Bay Road, above Kukupa, Akaroa, Banks Peninsula, Canterbury, H. D. Wilson BP1134; WELT 58664/A, Petrie Herbarium, Low Hills, Petichet Bay, Dunedin, Otago; WELT 58671, Near New Brighton (Christchurch), Canterbury, L. Cockayne.

CONSERVATION STATUS: Common in the South Island. Not threatened.

ETYMOLOGY: The epithet *fulvidus* refers to the leaves and branchlets being "somewhat tawny".

ILLUSTRATIONS: Poole & Adams (1994, p. 203).

Ozothamnus leptophyllus* subsp. *retortus (A. Cunn. ex DC.) Schönberger comb. et. stat. nov.

≡ *Cassinia retorta* A. Cunn. ex DC., Prodromus 6: 154 (1838).

Holotype: Hokianga river, A. Cunningham, Allan Cunningham's New Zealand Herbarium, K (photograph seen), fruiting piece on right-hand side labelled 64 with a tag, label below gives the number 447.

DESCRIPTION: Slightly straggling shrub, 80-150 cm tall, inner branches erect, outer branches sometimes decumbent or slightly prostrate, >5. Branchlets c. 13 per 5 cm branch at angle of c. 37°, sometimes descending or deflexed, 0.7-1.5(-2) mm diameter, clothed with very dense tomentum sometimes overlain with yellow exudates, new branchlets developing below old terminal inflorescence after fruiting. Leaves c. 8-18 per cm branchlet; lamina orientation >90°, lamina often strongly recurved, narrow-obovate, sometimes oblong, (2-)3-4(-5) × (1-)1.5-2 mm; apex rounded to obtuse; adaxial surface of young and mature leaves sparsely to densely covered with fine hairs; abaxial surface clothed with extremely dense white tomentum, not or sometimes sparsely to densely overlain with yellow exudates; margins strongly recurved; midvein very strongly evident on abaxial surface. Panicles slightly diffuse, often simple, rounded, c. 15(-35) mm diameter. Pedicels c. 2-6(-15) mm long. Capitula 6-11 per panicle, 7-7.5(-9) × 3-4.5(-5) mm. Outer involucral bracts 16-20, ovate to narrow-ovate, tawny green below, yellowish towards the tips; tips membranous, not spreading; abaxial surface densely hairy. Inner involucral bracts c. 7(-10), c. 5 × 1 mm, tips white, obtuse, radiating; abaxially moderately hairy. Receptacle scales 20-27, with erect white tips. Florets 12-18(-20) per capitulum. Corolla tube c. 3.5-4 mm long, lobes c. 0.8 × 0.45 mm. Style c. 3 mm long; style branches c. 1 mm. Achenes 1-1.5 × 0.5 mm; almost always glabrous, rarely with sparse glandular hairs. Pappus up to 4 mm long, slightly thickened at tips; apical cells c. 4; barbellae short, hardly spreading. Chromosome number: not known. FL (Dec)Jan-Mar, FT Jan-Apr.

DISTRIBUTION (Fig. 5.2 E): Auckland, Taranaki and Gisborne provincial areas as far south as latitude 40°30'S on the west coast and 38°S on the east coast. Coastal.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *retortus* is distinguished from other *Ozothamnus leptophyllus* taxa by very large capitula in small almost simple panicles,

comprising only a few, 6-11, capitula. The large capitula contain 12-18 and more florets. Floral characters, such as larger floral parts in great numbers and its more or less prostrate growth habit distinguishes *O. leptophyllus* subsp. *retortus* clearly from *O. leptophyllus* subsp. *fulvidus* and *O. leptophyllus* subsp. *leptophyllus* with which it shares most of the vegetative characters. *O. leptophyllus* subsp. *retortus* is distinguished from the *O. leptophyllus* subsp. *vauvilliersii* varieties, *O. leptophyllus* subsp. *albidus* and *O. leptophyllus* subsp. *amoenus* by having smaller, closer set leaves.

VARIATION: The colour and density of exudates on branchlets and leaves is variable in *O. leptophyllus* subsp. *retortus* according to geographic area. The plants in the extreme north of Auckland Province appear much more yellow than further south.

REPRESENTATIVE SPECIMENS: AK 116152, Coalmine Bay, Whangaparaoa Peninsula, North Auckland, J. H. Goulding; AK 136858, Great Mercury Island, Coromandel, A. E. Wrigth; AK 150358, Clifftop north of Waiiti Bay, Motukawanui Island, Cavalli Islands, D. J. Court ; AK 171622, Fraser's Landing, Aorangi Island, Poor Knights Islands, A. E. Wright 7356 ; AK 217747, Maunganui Bluff Beach, Tutamoe, North Auckland, A. E. Esler; AK 35661, South of Mitimiti, Maungataniwha, North Auckland, R. C. Cooper; AK 80172, Motukiekie Island, Bay of Island, R. E. Beever; AK 92909, sand dunes towards mouth of Aotea Harbour, South Auckland, P. Hynes; CANU 20791, Tokerau village, T. Dobson; CANU 38577, Te Werahi Beach, North Cape, North Auckland, I. Schönberger 105A; CANU 38578, Hooper Point, North Cape, North Auckland, I. Schönberger 106A; CANU 38590, Ninety Mile Beach, Hukatere fire lookout, North Auckland, North Auckland, I. Schönberger 108A; CHR 175597, Wood hill Forest, North Auckland, C. Smithies; CHR 184218, Mokohinau Island, Hauraki Gulf, North Auckland, G. L. Collett; CHR 208879, Waikawau Beach, South Auckland, A. P. Druce; CHR 225401, Opotiki, Maraenui, Gisborne, A. E. Esler; CHR 225547, Coromandel Islands, Motuwi, Hauraki Gulf, North Auckland, A. E. Esler; CHR 321444, Great Barrier Island, North Auckland, J, K. Bartlett; OTA 2435, Waikuku Beach, North Cape, North Auckland, G. T. S. Baylis; WELT 9738, Piha, North Auckland, W. R. B. Oliver.

CONSERVATION STATUS: Not threatened.

ETYMOLOGY: The epithet *retortus* refers to the leaves often being "bent back".

ILLUSTRATIONS: Salmon (1991, p. 66: 265); Poole & Adams (1994, p. 203), Eagle (1986, Vol. I: 202).

Ozothamnus leptophyllus* subsp. *vauvilliersii (Hombr. et Jacq.) Schönberger comb. et. stat. nov.

≡ *Ozothamnus vauvilliersii* Hombr. et Jacq. ex Decne in Voy. Pôle Sud pl. 5 (1843).

≡ *Cassinia vauvilliersii* (Hombr. et Jacq.) Hook.f., Flora Antarctica 1: 29 (1847).

Holotype: "Iles Auckland-Sud de la Nouvelle Zelande", M. Hombron 1841, P 179961 (photograph seen).

= *Olearia xanthophylla* Colenso, Trans. & Proc. N.Z. Inst. 20: 193 (1888).

Holotype: High Plains, Waimarino, on the west side of Mount Tongariro, H. Hill 1887, W. Colenso Herbarium, WELT 24144!

DESCRIPTION: Erect, round-topped shrub, 70-200 cm tall, main branches somewhat spreading, outer branches sometimes decumbent or prostrate, >5. Branchlets c. 7 per 5 cm branch at angle of c. 42°, never descending or deflexed, c. 1.6 mm diameter, clothed with moderate to dense tomentum overlain with pale yellow-green to deep orange-yellow exudates of varying density, new branchlets developing below old terminal inflorescence after fruiting. Leaves 8-10 per cm branchlet; orientation of young leaves c. 30°, mature leaves c. 60°, sometimes recurved; lamina narrow-obovate or elliptic to narrow-elliptic, 3.5-10 × 1.8-4 mm, apex obtuse or rounded, rarely acute or retuse; adaxial surface of young and mature leaves sparsely to densely covered with fine hairs; abaxial surface clothed with moderately dense to dense tomentum always overlain with pale yellow-green or bright yellow to orange-yellow exudates of varying density; young leaves sometimes glutinous; margins slightly to moderately recurved; midvein slightly to moderately evident on abaxial surface. Panicles dense to diffuse, sometimes several times compound, rounded, 15-45 mm diameter. Pedicels 0.4-3 mm long. Capitula 14-50 in less compound panicles, up to 100 in large compound panicles, 5-6.5 × 2.5-4 mm. Outer involucral bracts 9-14, ovate to broad-ovate, greenish brown below, often reddish, bright red or pink towards the tips; tips acute or with a small cream scarious erose apex, not or very slightly spreading; abaxial surface sparsely to densely hairy. Inner involucral bracts 9-15, 4-4.5 × 0.7-1 mm, tawny to pale

green, sometimes reddish towards the white, obtuse, radiating tips; abaxially sparsely to moderately hairy. Receptacle scales 5-17, with erect white tips. Florets c. 9-20 per capitulum. Corolla tube c. 2-3 mm long, lobes c. 0.4-0.7 × 0.3-0.4 mm. Style c. 1.8-2.8 mm long; style branches 0.5-0.8 mm. Achenes 0.6-1(-1.6) × 0.3-0.6 mm; twin hairs absent to dense; glandular hairs absent or sparse. Pappus hairs 3-3.5 mm long, moderately to distinctly thickened at tips; apical cells c. 4; barbellae short, hardly spreading. Chromosome number: not known. FL Nov-May, FT Dec-Jun.

DISTRIBUTION (Fig. 5.2 F): North Island, South Island, Stewart Island, Auckland Islands; from 37°S southwards. From montane and subalpine shrubland and grassland in the North Island and the Southern Alps down to sea level in South-Otago, Southland, Fiordland, Stewart Island, and the Auckland Islands.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *vauvilliersii* is distinguished from *O. leptophyllus* subsp. *fulvidus* by broader leaves, larger capitula and floral parts, and numerous receptacle scales among the florets. Its larger size, the presence of dense yellow exudates on branchlets and leaves, and the presence of numerous receptacle scales among the florets readily distinguish *O. leptophyllus* subsp. *vauvilliersii* from *O. leptophyllus* subsp. *amoenus*. *O. leptophyllus* subsp. *vauvilliersii* is distinguished from *O. leptophyllus* subsp. *albidus* by a less dense tomentum overlain with yellow exudates on leaves and branchlets, slightly smaller leaves, more slender pappus hairs that are spreading at the tips, and longer anther tails.

Key to the varieties of *Ozothamnus leptophyllus* subsp. *vauvilliersii*

1 Abaxial surface of leaves clothed with dense tomentum, white or pale yellowish-green ..... **var. *pallidus***

Abaxial surface of leaves clothed with moderately dense tomentum, more or less yellow *2*

2 Leaves 3.5-6(-7) × 1.8-2.5 mm, narrow-obovate or narrow-elliptic ..... **var. *collinus***

Leaves 4.5-10 × 2.5-4 mm, obovate or elliptic ..... **var. *vauvilliersii***

Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii

= *Cassinia fulvida* var. *montana* Allan, Flora N.Z. 1: 729 (1961).

Holotype: Arthur's Pass, Canterbury, Alt. 914 m, V. D. Zotov, 15.01.1936, CHR 9397!

= *Cassinia vauvilliersii* var. *serpentina* Cockayne et Allan, Trans. & Proc. N.Z. Inst. 56: 22 (1926).

Holotype: Mineral Belt Dun Mountain, L. Cockayne, L. Cockayne Herbarium 1930, Dec. 1916, AK 31977!

= *Cassinia rubra* Buchanan, Trans. & Proc. N.Z. Inst. 19: 216 (1887).

≡ *Cassinia vauvilliersii* var. *rubra* (Buchanan) Cheeseman, Manual N.Z. flora: 346 (1906).

No type material of *C. rubra* could be located.

DESCRIPTION: Erect, round-topped shrub, 70-200 cm tall, main branches somewhat spreading, outer branches sometimes decumbent or prostrate, >5. Branchlets c. 7 per 5 cm branch at angle of c. 42°, never descending or deflexed, c. 1.6 mm diameter, clothed with moderate tomentum always densely overlain with yellow to deep orange-yellow exudates, new branchlets developing below old terminal inflorescence after fruiting. Leaves 8-10 per cm branchlet; orientation of young leaves c. 30°, mature leaves c. 60°; lamina narrow-obovate or elliptic, 4.5-10 × 2.5-4 mm, apex obtuse or rounded, rarely acute or retuse; adaxial surface of young and mature leaves sparsely covered with fine hairs; abaxial surface clothed with moderately dense tomentum always densely overlain with yellow to deep orange-yellow exudates; margins slightly to moderately recurved; midvein slightly to moderately evident on abaxial surface. Panicles dense to diffuse, sometimes several times compound, rounded, 15-45 mm diameter. Pedicels 0.4-3 mm long. Capitula 14-50 in less compound panicles, up to 100 in large compound panicles, 5-6.5 × 2.5-4 mm. Outer involucral bracts 9-14, ovate to broad-ovate, greenish brown, often reddish, bright red or pink towards the tips; tips acute, not or very slightly spreading; abaxial surface sparsely to moderately hairy. Inner involucral bracts 9-15, 4-4.5 × 0.7-1 mm, tawny to pale green, sometimes reddish towards the white, obtuse, radiating tips; abaxially sparsely to moderately hairy. Receptacle scales 8-17, with erect white tips. Florets c. 9-20 per capitulum. Corolla tube c. 2-3 mm long, lobes c. 0.4-0.7 × 0.3-0.4 mm. Style c. 1.8-2.8 mm long; style branches 0.5-0.8 mm. Achenes 0.6-1(-1.6) × 0.3-0.6 mm; twin hairs absent to dense; glandular hairs absent or sparse. Pappus hairs 3-3.5 mm long, moderately to

distinctly thickened at tips; apical cells c. 4; barbellae short, hardly spreading. Chromosome number: not known. FL Nov-May, FT Dec-Jun.

DISTRIBUTION (Fig. 5.2 F): North Island, South Island, Stewart Island, Auckland Islands; from 37°S southwards. From montane and subalpine shrubland and grassland in the North Island and the Southern Alps down to sea level in South-Otago, Southland, Fiordland, Stewart Island, and the Auckland Islands.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii* is distinguished from *O. leptophyllus* subsp. *fulvidus* by larger, mainly broader leaves, larger capitula and floral parts, and numerous receptacle scales among the florets. Its usually larger size, the presence of yellow exudates on branchlets and leaves, and the presence of numerous receptacle scales among the florets readily distinguish *O. leptophyllus* subsp. *vauvilliersii* from *O. leptophyllus* subsp. *amoenus*. *O. leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii* is distinguished from *O. leptophyllus* subsp. *albidus* by a less dense tomentum overlain with yellow exudates on leaves and branchlets, slightly smaller leaves, more slender pappus hairs that are spreading at the tips, and longer anther tails.

VARIATION: *Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii* is very polymorphic and shows considerable geographic variation. The habit ranges from very tall upright shrubs from lower altitudes in the southern part of its range, to low, almost prostrate shrubs, on mountain ridges from the more northern parts of its range. Plants from Fiordland and Southland, as well as some specimens from slightly west of the Main Divide and from the North Island, were found to have red-tinged outer involucral bracts and correspond to Buchanan's (1887) description of *Cassinia rubra*. This character is not consistent within an otherwise homogenous population and also tends to vary seasonally. Exudate colour and density also vary considerably. Plants from Southland occasionally have leaves with retuse apices, a unique character within *Ozothamnus leptophyllus*. The general appearance ranges from very tall upright shrubs with large leaves from lower altitudes in the southern part of its range, to low, almost prostrate shrubs with smaller leaves on mountain ridges in the more northern parts. The small-leaved representatives of *O. leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii* have been previously recognised as a distinct taxon, *Cassinia fulvida* var. *montana* Allan. Plants of *Ozothamnus leptophyllus*

subsp. *vauvilliersii* var. *vauvilliersii* found on the Mineral Belt in the Sounds-Nelson Botanical District, and previously described as *Cassinia vauvilliersii* var. *serpentina* Cockayne et Allan, are distinguished by slightly darker exudates on leaves and branchlets and leaf size in the lower range of the leave size within *Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii*. These might be seen as an ecotype considering the unusual habitat.

REPRESENTATIVE SPECIMENS: SOUTH ISLAND: CANU 38564, Garibaldi Ridge, NW Nelson, A. D. Wilton (I. Schönberger 9A); CANU 38566, Otira Valley, Westland, R. McKenzie (I. Schönberger 13A); CANU 38568, Lake Wapiti, Fiordland, D. Glenny 7484 (I. Schönberger 17A); CANU 38571, Deer Spur Track, Mt Peel, Canterbury, I. Schönberger 35A; CANU 38574, Red Tarns, Mt Cook National Park, Canterbury, I. Schönberger 64A; CANU 38580, Cathedral Cave Walk Way, Catlins, Southland, I. Schönberger 118A; CANU 38588, Danseys Pass, Otago, D. Glenny 7704; CHR 131611, Near Sylvester Hut, NW Nelson, A. P. Druce; CHR 179235, Moraine of Douglas Glacier, Wesltand National Park, Westland, T. R. Fryer ; CHR 227458, Wet Jacket Arm, Oke Island at centre Fiordland, D. R. Given 72278; CHR 254091, Glencoe fan, Mt Cook National Park, South Canterbury, J. M. Wilson; WELT 58310, Raikaia Valley, Canterbury, T. Kirk; WELT 58341, Fox Glacier, Westland, E. M. Heine; WELT 58342, P[illegible] Range, L. Cockayne; WELT 58359, Bluff Hill, Southland, D. Petrie; WELT 58369, Dun Mountain, Nelson, E. K. Pickmere; WELT 9623, Mineral Belt, Nelson, W. R. B. Oliver; NORTH ISLAND: AK 107152, Napier-Taupo Road, Rangitaiki, Volcanic Plateau, K. Wood; AK 117894, Mount Ruapehu above Blythe Hut, Wellington, P. Hynes; AK 70504, Mt Pihanga, Tongariro National Park, Wellington, P. Hynes; CANU 38579, Mt Stratford ski field, Mt Egmont, Taranaki, A. Weiss and G. Eichinger (I. Schönberger 112A); CANU 38582, Pouaki Range, Taranaki, A. D. Wilton 99133 (I. Schönberger 148A); CHR 131190, Hauhungatahi Basin, Tongariro National Park, Wellington, A. P. Druce; CHR 153027, Summit of Arowhana, Raukumara Range, Gisborne, I. R. Fryer; CHR 165890, Mount Kakaramea, South Auckland, A. P. Druce; CHR 192499, Mount Kaiparoro, North Wairarapa, Wellington, A. P. Druce; CHR 209488, South West of Takapari, South Ruahine Range, Hawkes Bay, A. P. Druce; WELT 58344, Mt Egmont, north slopes, Taranaki, M. Sutherland; STEWART ISLAND: CANU 38585, Mason Bay, I. Breitwieser and R. Vogt 2120; CHR 220784, Fern Gully, L. R. Stemmer; CHR 355340, Rakeahua

Valley, H. D. Wilson 789-652; WELT 58336, Stewart Island, T. Kirk; AUCKLAND ISLANDS: BM 602394, J. D. Hooker; CHR 238611, Tucker Point, W. H. Dawbin; CHR 323165, Behind Terror Cove, D. R. Given; CHR 437299, Hooker Hills, W. R. Sykes; CHR 88846, Port Ross, Bog at Lookout, N. T. Moar.

CONSERVATION STATUS: Not threatened.

ETYMOLOGY: The epithet *vauvilliersii* commemorates Jean Francois Vauvilliers (1737-1801).

ILLUSTRATIONS: Hombron & Jacquinot (1843-1853, Plate 5); Wilson & Galloway (1993, p. 207: 108b); Poole & Adams (1994, p.203); Salmon (1991, p.213: 889); Salmon (1992, p. 35: 24, 25); Eagle (1986, Vol. II: 263).

Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *collinus Schönberger var. nov.
Ab varietatibus aliis *Ozothamni leptophylli* subsp. *vauvilliersii* foliis angustis, 1.8-2.5 mm latis differt.
Differing from the other varieties of *Ozothamnus leptophyllus* subsp. *vauvilliersii* by its narrow, 1.8-2.5 mm wide leaves.
Holotype: Cass Field Station, Canterbury, Alt. 570 m, G.J. Houliston, 22.03.2002, CANU; isotypes: CHR, WELT.
= *Cassinia fulvida* Hook.f., Handbook N.Z. flora: 145 (1864) pro parte.
Holotype: Canterbury?, Lyall, K (photograph seen), flowering piece on right-hand side top corner of sheet.

DESCRIPTION: Slightly straggling shrub, 80-150 cm tall, main branches somewhat spreading, >5. Branchlets c. 8 per 5 cm branch at angle of c. 42°, never descending or deflexed, c. 1.5 mm diameter, clothed with moderate tomentum always overlain with bright yellow coloured exudates, new branchlets developing below old terminal inflorescence after fruiting. Leaves c. 8 per cm branchlet; orientation of young leaves c. 20-30°, mature leaves c. 60°; lamina narrow-obovate sometimes narrow-elliptic, 3.5-6(-7) × 1.8-2.5 mm; apex obtuse to acute sometimes rounded; adaxial surface of young and mature leaves glabrous to sparsely covered with fine hairs; abaxial surface clothed with moderately dense

tomentum always densely overlain with bright yellow exudates; margins slightly to moderately recurved; midvein slightly to moderately evident on abaxial surface. Panicles dense, rounded, 15-30 mm diameter. Pedicels c. 1 mm long. Capitula c. 10-30 per panicle, 5-6 × 2-4 mm. Outer involucral bracts c. 10, ovate, greenish brown below, sometimes reddish towards the tips; tips acute not or very slightly spreading; abaxial surface sparsely to moderately hairy. Inner involucral bracts c. 9, 3.5-4 × 0.7-1 mm, tawny to pale green, with radiating obtuse white tips; abaxially glabrous to sparsely hairy. Receptacle scales 5-15, with erect white tips. Florets c. 7-15 per capitulum. Corolla tube c. 2-3 mm long, lobes c. 0.6 × 0.4 mm. Style c. 1.8-2.8 mm long; style branches 0.5-0.8 mm. Achenes 0.6-1 × 0.3-0.6 mm; twin hairs absent to dense; glandular hairs absent or sparse. Pappus hairs 2.5-3.5 mm long, moderately to distinctly thickened at tips; apical cells c. 4; barbellae short, hardly spreading. Chromosome number: $2n = 26$-28. FL (Nov)Jan-Apr, FT Jan-Jun.

DISTRIBUTION (Fig. 5.2 G): South Island east of the Main Divide from latitude 42°30'S southwards on the mountain ranges of Canterbury, Otago and Southland. Montane shrubland and grassland, descending lowland in the southern part of its range.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *collinus* and *O. leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii* are very similar in habit and overall appearance. *O. leptophyllus* subsp. *vauvilliersii* var. *collinus* is distinguished from *O. leptophyllus* subsp. *vauvilliersii* var. *vauvilliersii* by narrower leaves that resemble in size and shape the leaves of *O. leptophyllus* subsp. *fulvidus* from which it is distinguished by a more compact habit, smaller panicles with larger capitula, and more receptacle scales among the florets.

REPRESENTATIVE SPECIMENS: CANU 38567, Upper Cave Stream between West Coast Rd. and Helicopter Hill, R. McKenzie (I. Schönberger 15A); CANU 38589 Gorgeburn Valley, Eyre Mts, Southland, R. McKenzie (I. Schönberger 89A); CHR 518399, Poverty Flat, Mt White Road, Canterbury, M. Dawson G382/96; WELT 58366, Mt Oxford, Canterbury, E. M. Heine; WELT 58672, Clarence Valley, Marlborough, T. Kirk; WELT 9752, Mt Isobel, Canterbury, W. R. B. Oliver; WELTU 16016, Porters Pass, Canterbury, I. Breitwieser 986 & R. W. Vogt.

CONSERVATION STATUS: Not threatened.

ETYMOLOGY: The epithet *collinus* means "from the hills" and refers to the distribution of the variety.

Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *pallidus (Allan) Schönberger comb. nov.

≡ *Cassinia vauvilliersii* var. *pallida* Allan, Flora N.Z. 1: 728 (1961).

Holotype: Upper Awatere, Robinson Creek; H. H. Allan, 21.03.1951, CHR 87404!

DESCRIPTION: Slightly straggling shrub, 80-150 cm tall, main branches somewhat spreading, >5. Branchlets c. 6 per 5 cm branch at angle of c. 50°, never descending or deflexed, c. 1.6 mm diameter, clothed with dense tomentum, not or very sparsely overlain with pale yellow-green coloured exudates, new branchlets developing below old terminal inflorescence after fruiting. Leaves c. 6-8 per cm branchlet; orientation of young leaves c. 20-30°, mature leaves c. 60° sometimes recurved; lamina narrow-obovate, 4.5-8(-10) × 2-3.5 mm, apex obtuse or rounded, sometimes acute; adaxial surface of young and mature leaves densely covered with fine hairs; abaxial surface clothed with dense white tomentum very sparsely overlain with pale yellow-green exudates; young leaves glutinous; margins slightly to moderately recurved; midvein slightly to moderately evident on abaxial surface. Panicles dense, rounded, 30-45 mm in diameter. Pedicels 0.5-4 mm long. Capitula 30-50 per panicle, 5 × 3-4 mm. Outer involucral bracts c. 10, ovate, greenish brown below, often reddish, bright red or pink towards the tips; tips acute or scarious erose, very slightly spreading; abaxial surface sparsely to moderately hairy. Inner involucral bracts 9-15, 4 × 1 mm, pale green, sometimes reddish towards the radiating obtuse white tips, abaxially sparsely to moderately hairy. Receptacle scales c. 15 with erect white tips. Florets c. 15 per capitulum. Corolla tube c. 2.5-3 mm long, lobes c. 0.5 × 0.35 mm. Style c. 2.5 mm long; style branches 0.5-0.8 mm. Achenes 0.6-1.3 × 0.4-0.6 mm; twin hairs absent to dense; glandular hairs absent or sparse. Pappus hairs 3-3.5 mm long, moderately to distinctly thickened at tips; apical cells c. 4; barbellae short, hardly spreading. Chromosome number: $2n = 26$-28. FL (Nov)Jan-Apr(May), FT Jan-Jun.

DISTRIBUTION (Fig. 5.2 H): South Island, replaces *Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *collinus* between 41°S and 42°30'S in montane shrubland and grassland.

Both varieties form more or less regional populations within the range of *O. leptophyllus* subsp. *vauvilliersii* and occur in similar habitats.

RECOGNITION: *Ozothamnus leptophyllus* subsp. *vauvilliersii* var. *pallidus* is distinguished from the other *vauvilliersii* varieties by a denser tomentum on leaves and branchlets, which is overlain by a smaller amount of less brightly coloured pale yellowish green exudates. Young leaves and branchlets are often glutinous. Dense, fine, white hairs on the adaxial surface of the leaves give the plants a pale green appearance. The tomentum of the abaxial surface of the leaves and branchlets is less dense than that of *O. leptophyllus* subsp. *albidus*, which also lacks bright yellow exudates in large quantities. Smaller leaves with usually rounded or obtuse apices also distinguish *O. leptophyllus* subsp. *vauvilliersii* var. *pallidus* from *O. leptophyllus* subsp. *albidus*.

REPRESENTATIVE SPECIMENS: AK 255621, c. 1.5 km north-east of Island Saddle north side of road, Molesworth, Marlborough, E. K. Cameron; CANU 38565, West-end of St James Walkway, Lewis Pass, N Canterbury, I. Schönberger 11A; CHR 117686, Upper D'Urville River, Nelson Lakes National Park, Nelson, M. J. A. Simpson 4280; CHR 182033, St. Arnaud Range, Nelson, N. C. Lambrechtsen; CHR 22713, Shale Peak, Amuri, Marlborough, V. D. Zotov; CHR 51272, Lower slopes of Molesworth Hill, Marlborough, H. H. Allan; CHR 518398, Near St James Walkway, Lewis Pass, N Canterbury, M. Dawson 923/96; WELT 9764, Tophouse, Nelson, W. R. B. Oliver.

CONSERVATION STATUS: Not threatened.

ETYMOLOGY: The epithet *pallidus* refers to the "pale" appearance of leaves and branchlets of this subspecies.

ILLUSTRATIONS: Salmon (1992, p.34: 23).

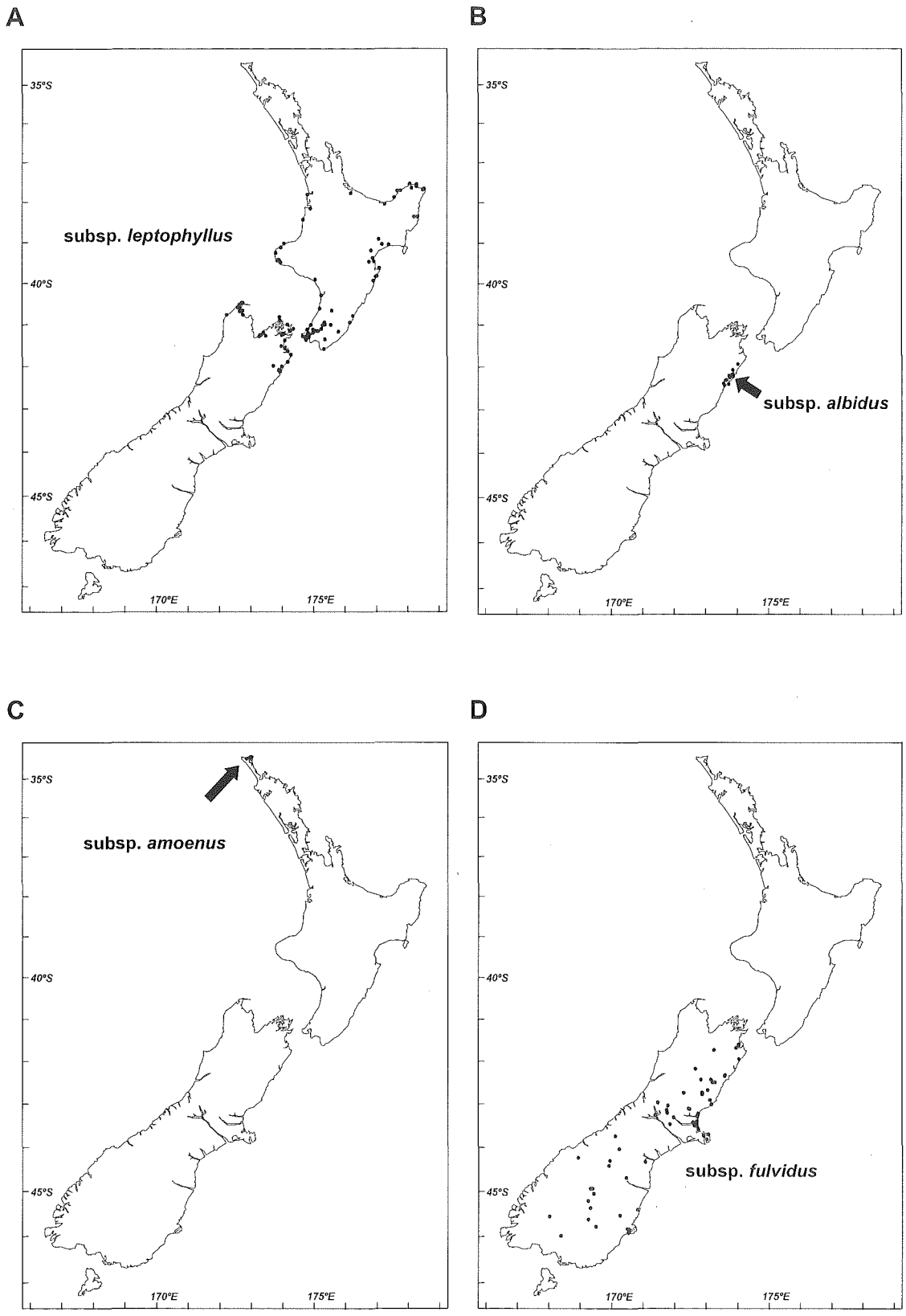


Fig. 5.2 see caption on page 282.

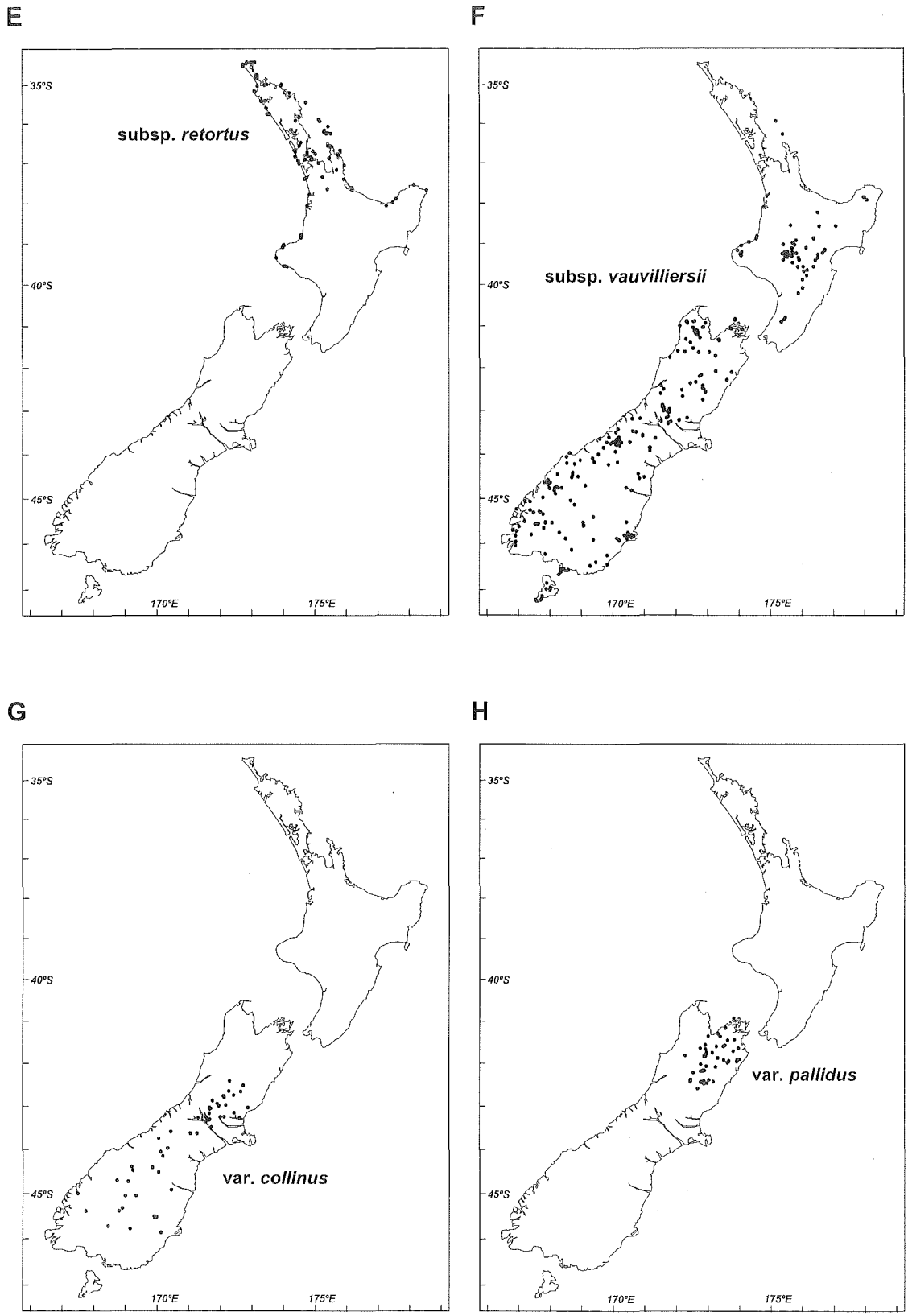


Fig. 5.2 Distributions of the subspecies and varieties of *Ozothamnus leptophyllus*. **A**, subsp. *leptophyllus*; **B**, subsp. *albidus*; **C**, subsp. *amoenus*; **D**, subsp. *fulvidus*; **E**, subsp. *retortus*; **F**, subsp. *vauvilliersii*; **G**, var. *collinus*; **H**, var. *pallidus*.

Acknowledgements

To my supervisor, Dr Josephine Ward, I direct special thanks and appreciation for her guidance and tremendous patience. I gratefully acknowledge her invaluable advice and support.

I am equally grateful to my associate supervisor, Dr Ilse Breitwieser, not only for assistance, support and help with my research, but also for being such a good friend and making time for me and my problems whenever I needed it.

Thanks to Dr Peter Lockhart and his team at Massey University, Palmerston North, for instructing me in the AFLP technique.

I am grateful to Dr Hazel Chapman for making lab facilities and equipment available to me during the molecular part of my work and for her support and encouragement.

I gratefully acknowledge the assistance and technical advice of Beth Robson. In particular I would like to thank her for her patience in teaching me lab techniques and for her hands-on help in the lab.

Thanks to Anthony Mitchell and Hazel Broadbent for their advice and help with my molecular work.

I am grateful to Steve Wagstaff for supplying me with a gel rig and plates to run polyacrylamide gels.

Assistance with fieldwork was provided by a number of people. Thanks to Anthony Mitchell, Tim Jenkins, Robert McKenzie, David Glenny, Angelika Weiss and Georg Eichinger.

I am grateful to the many colleagues and friends who have provided specimens and information, including Robin Smith (Percy's Reserve, Wellington), Michael Bayly, Phil Garnock-Jones, Kerry Ford, Robert McKenzie, Aaron Wilton, David Glenny, Josephine Ward, Barbara Brown, Ilse Breitwieser, Rainer Vogt, and Andy McCall. I would like to express my special gratitude to my friends, colleagues and fellow students and for their support, encouragement and assistance throughout this thesis. Special thanks go to Gary Houliston and Terry Thomsen for stimulating discussions and critically reading various parts of the text. I also thank Kerry Ford and David Glenny for their expert advice and insights.

Thanks to the staff of the Department of Plant and Microbial Sciences at the University of Canterbury, for technical assistance and advice received over the course of the investigation, and for making my stay at the University of Canterbury very enjoyable. Thanks to all, especially to Maggie Tisch, for making me feel welcome and "at home" in the department. The following provided support and assistance in specific ways: Neil Andrews provided advice and guidance on the SEM technique; Matt Walters took most of the photographs, and assembled and printed the figures for this thesis; Manfred Ingerfeld provided assistance with illustrations and ordered herbarium specimens; Graeme Young helped with all computer problems; Dave Conder and Bruce Boon tended the cultivated plants; Maggie Tisch, Shirl Jones and Reijel Gardiner helped in various ways and were there whenever I needed them.

I would like to thank Chris Frampton and Aaron Wilton for discussion and advice on statistics. Special thanks go to Aaron Wilton who made his S-Plus library available to me, and even added additional routines to make the analysis of my data easier.

My thanks to the curators at CHR, WELT, AK, OTA, BM, K, and P for providing loans or photographs of specimens. The efficiency and speed of Phillipa Scott in providing loans of specimens from WELT is gratefully acknowledged, as is the assistance of Jeff Fox in giving permission to use unpublished information on typification in *Cassinia*.

Finally, I would like to thank my partner Anthony Mitchell for his continual support, encouragement and assistance throughout this thesis. The completion of this thesis would not have been possible without his help. Anthony, thank you for all your patience and support. Your help was invaluable.

This work was made possible by financial support from the Deutscher Akademischer Austauschdienst DAAD (German Academic Exchange Service) and the University of Canterbury Doctoral scholarship.

References

Aggarwal, R. K., Brar, D. S., Nandi, S., Huang, N. & Khush, G. S. 1999: Phylogenetic relationships among *Oryza* species revealed by AFLP markers. *Theoretical and Applied Genetics* **98**: 1320-1328.

Allan, H. H. 1961: Flora of New Zealand. Vol. I. Government Printer, Wellington.

Anderberg, A. A. 1989: Phylogeny and reclassification of the tribe Inuleae (Asteraceae). *Canadian journal of botany* **67**: 2277-2296.

Anderberg, A. A. 1991: Taxonomy and phylogeny of the tribe Gnaphalieae (Asteraceae). *Opera Botanica* **104**: 1-195.

Andersson, , L. 1990: The driving force: species concepts and ecology. *Taxon* **39**: 375-382.

Antonius, K. & Nybom, H. 1994: DNA fingerprinting reveals significant amounts of genetic variation in a wild raspberry *Rubus idaeus* population. *Molecular Ecology* **3**: 177-180.

Arens, P., Coops, H., Jansen, J. & Vosman, B. 1998: Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. *Molecular Ecology* **7**: 11-18.

Babcock, E. B. 1947: The genus *Crepis*, Part I, The Taxonomy, Phylogeny, Distribution, and Evolution of *Crepis*. University of California Press, Berkeley.

Banks, J. S., Solander, D. C., Parkinson, S., Museum, B. & Editions, A. H. 1980-1990: Banks' Florilegium. 6/100 edit. Alecto Historical Editions in association with the British Museum (Natural History), London.

Bayer, R. J., Puttock, C. F. & Kelchner, S. A. 2000: Phylogeny of South African Gnaphalieae (Asteraceae) based on two noncoding chloroplast sequences. *American journal of botany* **87**: 259-272.

Bayer, R. J. & Starr, J. R. 1998: Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the trnL intron and trnL/trnF intergenic spacer. *Annals of the Missouri Botanical Garden* **85**: 242-256.

Beismann, H., Barker, J. H. A., Karp, A. & Speck, T. 1997: AFLP analysis sheds light on distribution of two *Salix* species and their hybrids along a natural gradient. *Molecular Ecology* **6**: 989-993.

Bemis, W. P., Rhodes, A. M., Whitaker, T. W. & Carmer, S. G. 1970: Numerical taxonomy applied to *Cucurbita* relationships. *American journal of botany* **57**: 404-412.

Benson, L. 1962: Plant taxonomy methods and principles. The Ronald Press Company, New York.

Bentham, G. 1866: Flora Australiensis. Vol. 3. Reeve, London.

Bentham, G. 1873: Compositae. *In*: Bentham, G. & Hooker, J. D. (eds.). Genera Plantarum Vol. II, pp. 163-533. Lovell Reeve & Co., London.

Bergoth, E. E. 1927: Hemiptera Heteroptera from New Zealand. *Transactions of the New Zealand Institute* **57**: 671-684.

Betts, M. W. 1918: Notes on the autecology of certain plants of the Peridotite Belt, Nelson. Part I Structure of some of the plants (No. 1). *Transactions of the New Zealand Institute* **50**: 230-243.

Betts, M. W. 1919: Notes on the autecology of certain plants of the Peridotite Belt, Nelson. Part I Structure of some of the plants (No. 2). *Transactions of the New Zealand Institute* **51**: 136-156.

Betts, M. W. 1920: Notes on the autecology of certain plants of the Peridotite Belt, Nelson. Part I Structure of some of the plants (No. 3). *Transactions of the New Zealand Institute* **52**: 276-314.

Betts, M. W. 1920b: Notes from Canterbury College Mountain Biological Station, Cass (No. 7) The rosette plants, Part 1. *Transactions of the New Zealand Institute* **52**: 253-275.

Black-Samuelsson, S., Eriksson, G., Gustafsson, L. & Gustafsson, P. 1997: RAPD and morphological analysis of the rare plant species *Vicia pisiformis* (Fabaceae). *Botanical journal of the Linnean Society* **61**: 325-343.

Bock, W. L. 1986: Species concepts, speciation, and macroevolution. *In*: Iwatsuki, K., Raven, P. H. & Bock, W. J. (eds.). Modern aspects of species, pp. 31-57. Univ. Tokyo Press, Tokyo.

Bohlmann, F., Mahanta, P. K., Suwita, A., Natu, A. A., Zdero, C., Dorner, W., Ehlers, D. & Grenz, M. 1977: Neue sesquiterpenlactone und andere Inhaltsstoffe aus Vertretern der Eupatorium-Gruppe. *Phytochemistry* **16**: 1973-1981.

Breitwieser, I. 1993: Comparative leaf anatomy of New Zealand and Tasmanian Inuleae (Compositae). *Botanical journal of the Linnean Society* **111**: 183-209.

Breitwieser, I., Glenny, D., Thorne, A. & Wagstaff, S. J. 1999: Phylogenetic relationships in Australasian Gnaphalieae (Compositae) inferred from ITS sequences. *New Zealand journal of botany* **37**: 399-412.

Breitwieser, I. & Sampson, F. B. 1997a: Pollen characteristics of New Zealand Gnaphalieae (Compositae) and their taxonomic significance - 1 LM and SEM. *Grana* **36**: 65-79.

Breitwieser, I. & Sampson, F. B. 1997b: Pollen characteristics of New Zealand Gnaphalieae (Compositae) and their taxonomic significance - 2 TEM. *Grana* **36**: 80-95.

Breitwieser, I. & Ward, J. M. 1993: Systematics of New Zealand Inuleae (Compositae-Asteraceae)-3. Numerical phenetic analysis of leaf anatomy and flavonoids. *New Zealand journal of botany* **31**: 43-58.

Breitwieser, I. & Ward, J. M. 1997: Transfer of *Cassinia leptophylla* (Compositae) to *Ozothamnus*. *New Zealand journal of botany* **35**: 125-128.

Bremer, B. 1991: Restriction data from cpDNA for phylogenetic reconstruction: is there only one accurate way of scoring? *Plant Systematics and Evolution* **175**: 39-54.

Briggs, D. & Walters, S. M. 1997: Plant variation and evolution. 3rd edit. Cambridge University Press, Cambridge.

Brown, R. 1817: Observation on the natural family of plants called Compositae. *Transactions of the Linnean Society of London* **12**: 78-142.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998: Multilocus and single-locus DNA fingerprinting. *In*: Hoelzel, A. R. (ed.). Molecular Genetic Analysis of Populations, pp. 287-336. Oxford University Press, Oxford, UK.

Brummitt, R. K. & Powell, C. E. 1992: Authors of plant names. Kew, Royal Botanic Gardens, London.

Brummitt, R. K. 1985: Lectoparatypes? *Taxon* **34**: 501-502.

Bryant, G. 1992: Gardens for free: a propagation handbook for New Zealanders. David Bateman.

Buchanan, J. 1887: On some native plants, Art. XXIX. *Transactions of the New Zealand Institute* **19**: 213-216.

Burger, W. C. 1975: The species concept in *Quercus*. *Taxon* **24**: 45-50.

Candolle, A. P. d. 1837: Prodromus systematis naturalis regni vegetabilis. Vol. VI. Treuttel & Würtz, Paris.

Carse, H. 1930: Botanical Notes, New Species and New Hybrids. *Transactions of the New Zealand Institute* **60**: 571-574.

Cassini, H. 1828: Uchite, *Euchiton*. *Dictionaire des sciences naturelles* **56**: 214-218.

Cheeseman, T. F. 1897: On some plants new to New Zealand flora, Art. XXXI. *Transactions of the New Zealand Institute* **29**: 390-393.

Cheeseman, T. F. 1906: Manual of the New Zealand flora. Government Printer, Wellington.

Cheeseman, T. F. 1907: Contributions to a fuller knowledge of the flora of New Zealand: No 1, Art. XXXVIII. *Transactions of the New Zealand Institute* **39**: 439-450.

Cheeseman, T. F. 1914: Illustrations of the New Zealand Flora. Vol. I. Government Printer, Wellington.

Cheeseman, T. F. 1925: Manual of the New Zealand Flora. 2nd edit (Oliver, ed.). Government Printer, Wellington.

Chiapella, J. 2000: The *Deschampsia cespitosa* complex in central and northern Europe: a morphological analysis. *Botanical journal of the Linnean Society* **134**: 495-512.

Clark, A. F. 1935: The winter-moth (*Hybernia indolcis* Walk.). *New Zealand journal of science and technology* **17**: 541-549.

Clausen, J. 1951: Stages in the evolution of plant species. Cornell Univ. Press, Ithaca, New York.

Clevinger, J. A. & Panero, J. L. 2000: Phylogenetic analysis of Silphium and subtribe Engelmanniinae (Asteraceae: Heliantheae) based on ITS and ETS sequence data. *American journal of botany* **87**: 565-572.

Cockayne, L. 1906: Notes on the subalpine scrub of Mount Fyffe (Seaward Kaikouras), Art. XLVI. *Transactions of the New Zealand Institute* **38**: 361-374.

Cockayne, L. 1910: New Zealand plants and their story. Government Printer, Wellington.

Cockayne, L. 1918: Notes on New Zealand Floristic Botany, including Descriptions of New Species, &c. (No.3), Art. XVII. *Transactions of the New Zealand Institute* **50**: 161-191.

Cockayne, L. 1928: The Vegetation of New Zealand. 2nd edit. Engelmann Press, Leipzig.

Cockayne, L. & Allan, H. H. 1926a: A proposed new botanical district for the New Zealand region. *Transactions of the New Zealand Institute* **56**: 19-20.

Cockayne, L. & Allan, H. H. 1926b: Notes on New Zealand floristic botany, including descriptions of new species, &c. (No. 4). *Transactions of the New Zealand Institute* **56**: 21-33.

Cockayne, L. & Allan, H. H. 1934: Annotatea list of groups of wild hybrids in the New Zealand Flora. *Annals of Botany* **38**: 1-55.

Colenso, W. 1888: On new phaenogamic plants of New Zealand, Art. XXVII. *Transactions of the New Zealand Institute* **20**: 188-211.

Colenso, W. 1890: A description of some newly-discovered phaenogamic plants, being a further contribution towards the making-known the botany of New Zealand, Art. LIX. *Transactions of the New Zealand Institute* **22**: 459-492.

Connor, H. E. 1967: Interspecific hybrids in *Chionochloa* (Gramineae). *New Zealand journal of botany* **5**: 3-16.

Connor, H. E. 1991: *Chionochloa* Zotov (Gramineae) in New Zealand. *New Zealand journal of botany* **29**: 219-282.

Crawford, D. J. 1990: Plant Molecular Systematics: Macromolecular Approaches. John Wiley & Sons, New York.

Crawford, D. J., Kimball, R. T. & Tadesee, M. 2001: The generic placement of a morphologically enigmatic species in Asteraceae: evidence from ITS sequences. *Plant systematics and evolution* **228**: 63-69.

Cresswell, A., Sackville Hamilton, N. R., Roy, A. K. & Viegas, B. M. F. 2001: Use of amplified fragment length polymorphism markers to assess genetic diversity of *Lolium* species from Portugal. *Molecular Ecology* **10**: 229-241.

Crovello, T. J. 1968a: Different concepts of relevance in a numerical taxonomic study. *Nature* **218**: 492.

Crovello, T. J. 1968b: The effect of missing data and two sources of character values on a phenetic study of the willows of California. *Madrono* **19**: 301-315.

Crovello, T. J. 1968c: The effect of change of number of OTU's in a numerical taxonomic study. *Brittonia* **20**: 346-367.

Crovello, T. J. 1970: Analysis of character variation in ecology and systematics. *Annual review of ecology and systematics* **1**: 55-98.

Cunningham, A. 1837: *In*: Candolle, A. P. d. (ed.). Prodromus Systematis Naturalis Regni Vegetabilis. Vol. VI. Treuttel & Würtz, Paris.

Cunningham, A. 1839: Florae insularum Novae Zelandiae precursor; or a specimen of the botany of the islands of New Zealand, Art. XVII. *Annals of Natural History; or Magazine of Zoology, Botany, and Geology* **2**: 125-131.

Cunningham, G. H. 1927a: "Natural control" of weeds and insects by fungi. *New Zealand journal of agriculture* **34**: 242-251.

Cunningham, G. H. 1927b: The Polyporaceae of New Zealand. *Transactions of the New Zealand Institute* **58**: 202-250.

Cunningham, G. H. 1965: Polyporaceae of New Zealand. *New Zealand Department of Scientific and Industrial Research bulletin* **164**: 304 pp.

Davis, P. H. 1978: The moving staircase - a discussion on taxonomic rank and affinity. *Notes from the Royal Botanical Garden Edinburgh* **36**: 325-340.

Davis, P. H. & Heywood, V. H. 1963: Principles of angiosperm taxonomy. Oliver & Boyd, London.

Dawson, M. I. & Beuzenberg, E. J. 2000: Contributions to a chromosome atlas of the New Zealand flora - 36. Miscellaneous families. *New Zealand journal of botany* **38**: 1-23.

Dawson, M. I., Ward, J. M., Groves, B. E. & Hair, J. B. 1993: Contributions to a chromosome atlas of the New Zealand flora - 32. *Raoulia* (Inuleae-Compositae (Asteraceae). *New Zealand journal of botany* **31**: 97-106.

Diment, J. A., Humphries, C. J., Press, J. R., Shaughnessy, E. & Newington, L. 1987: Catalogue of the natural history drawings commissioned by Joseph Banks on the Endeavour voyage 1768-1771 held in the British Museum (Natural History) Part 2: Botany: Brazil, Java, Madeira, New Zealand, Society Islands and Tierra del Fuego. Bulletin of the British Museum (Natural History). Historical Series Volume 12 (Complete). Vol. 2. British Museum (Natural History), London.

Dingley, J. M. 1969: Records of plant diseases in New Zealand. *New Zealand Department of Scientific and Industrial Research bulletin* **192**: 298 pp.

Doyle, J. J. 1991: DNA protocols for plants. *In*: Hewitt, G. M., Johnston, A. W. B. & Young, J. P. W. (eds.). Molecular Techniques in Taxonomy, pp. 283-285. Springer-Verlag, Berlin.

Doyle, J. J. & Doyle, J. L. 1987: A rapid isolation procedure from small quantities of fresh leaf tissue. *Phytochemistry Bulletin* **19**: 11-15.

Druce, A. P. 1993: Indigenous vascular plants of New Zealand (9th revision), Unpublished checklist held at Landcare Research, Lincoln, New Zealand.

Drury, D. G. 1970: A fresh approach to the classification of the genus *Gnaphalium* with particular reference to the species present in New Zealand (Inuleae-Compositae). *New Zealand journal of botany* **8**: 222-248.

Duncan, T. & Baum, B. R. 1981: Numerical phenetics: its uses in a botanical systematics. *Annual review of ecology and systematics* **12**: 387-404.

Dunn, G. & Everitt, B. S. 1982: An introduction to mathematical taxonomy. Cambridge University Press, Cambridge.

D'Urville, D. J. S. C. 1841-1854: Voyage au Pole Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zélée, exécuté par ordre du roi pendant les années 1837-1838-1838-1840 sous le commandement de M.J. Dumont d'Urville. Gide et J. Baudry, Paris.

Eagle, A. 1986: Eagle's trees and shrubs of New Zealand. Auckland, Collins.

Edgar, E. 1986: *Poa* L. in New Zealand. *New Zealand journal of botany* **24**: 425-503.

Ehrlich, P. R. & Ehrlich, A. H. 1967: The phenetic relationships of the butterflies I. Adult taxonomy and the nonspecificity hypothesis. *Systematic Zoology* **16**: 301-317.

Evans, K. M., Newbigin, E. & Ladiges, P. Y. 2002: An investigation of the genetic variation in *Banksia integrifolia* (Proteacea) by the use of the AFLP technique. *Australian systematic botany* **15**: 9-7.

Eyles, A. C. & Carvalho, J. C. M. 1995: Further endemic new genera and species of Mirinae (Hemiptera: Miridae) from New Zealand. *New Zealand journal of zoology* **22**: 49-90.

Farris, J. S. 1969: On the cophenetic correlation coefficient. *Systematic Zoology* **18**: 279-285.

Ferris, G. F. & Klyver, F. D. 1932: Report upon a collection of Chermidae (Homoptera) from New Zealand. *Transactions of the New Zealand Institute* **63**: 34-61.

Fisher, F. J. F. 1965: The alpine *Ranunculi* of New Zealand. Government Printer, Wellington.

Follett, J. M. & Foggo, M. N. 1981: The propagation by cuttings of three indigenous mountain shrub species. *Annual Journal, Royal New Zealand Institute of Horticulture* **9**: 49-56.

Forster, G. 1786: Florulae insularum australium prodomus. Dietrich, Göttingen.

Francisco-Ortega, J., Newbury, H. J. & Ford-Lloyd, B. V. 1993: Numerical-analyses of RAPD data highlight the origin of cultivated tagasaste (*Chamaecytisus proliferus* ssp *palmensis*) in the Canary Islands. *Theoretical and Applied Genetics* **87**: 264-270.

Garnock-Jones, P. J. 1986: A new status for the New Zealand mousetail (*Myosurus*, Ranunculaceae). *New Zealand journal of botany* **24**: 351-354.

Garnock-Jones, P. J. & Langer, H. J. 1980: *Parahebe catarractae*. *New Zealand journal of botany* **18**: 285-298.

Gatt, M. K., Hammett, K. R. W. & Murray, B. G. 2000: Molecular phylogeny of the genus *Dahlia* Cav. (Asteraceae, Heliantheae-Coreopsidinae) using sequences derived from the internal transcribed spacers of nuclear ribosomal DNA. *Botanical journal of the Linnean Society* **133**: 229-239.

Giannasi, N., Thorpe, R. S. & Malhotra, A. 2001: The use of amplified fragment length polymorphism in determining species trees at fine taxonomic levels: analysis of a medically important snake, *Trimeresurus albolabris*. *Molecular Ecology* **10**: 419-426.

Glenny, D. 1997: A revision of the genus *Anaphalioides* (Asteraceae: Gnaphalieae). *New Zealand journal of botany* **35**: 451-477.

Glenny, D. & Wagstaff, S. 1997: Evolution and biogeography of New Zealand *Anaphalis* (Asteraceae, Gnaphalieae) inferred from rDNA sequences. *New Zealand journal of botany* **35**: 441-449.

Gottlieb, L. D. 1984: Genetics and morphological evolution in plants. *The American Naturalist* **123**: 681-709.

Gower, J. C. 1966: Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53**: 325-338.

Gower, J. C. 1971: A general coefficient of similarity and some of its properties. *Biometrics* **27**: 857-871.

Grant, V. 1957: The plant species in theory and practice. *In*: Mayr, E. (ed.). The species problem, pp. 39-80. American Association for the Advancement of Science, Washington, D.C.

Greuter, W., McNeill, J., Barrie, F. R., Burdet, H. M., Demoulin, V., Filgueiras, D. H., Nicholson, D. H., Silva, P. C., Skog, J. E., Trehane, P., Turland, N. J. & Hawksworth, D. L., eds. 2000: International code of botanical nomenclature (Saint Louis code). Königstein: Koeltz Scientific Books.

Harris, S. A. 1999: RAPDs in systematics - a useful methodology? *In*: Hollingsworth, P. M., Bateman, R. M. & Gornall, R. J. (eds.). Molecular Systematics, Plant and Evolution, pp. 221-228. Taylor and Francis, London.

Harrison, R. A. 1959: Acalypterate Diptera of New Zealand. *New Zealand Department of Scientific and Industrial Research bulletin* **128**: 373 pp.

Heenan, P. B., de Lange, P. J. & Wilton, A. D. 2001: *Sophora* (Fabaceae) in New Zealand: taxonomy, distribution, and biogeography. *New Zealand journal of botany* **39**: 17-53.

Heiser, C. B. J., Soria, J. & Burton, D. L. 1965: A numerical taxonomic study of *Solanum* species and hybrids. *American Naturalist* **99**: 471-488.

Heslop-Harrison, J. 1960: New concepts in flowering-plant taxonomy. Heinemann, London.

Heywood, V. H. 1967: Variation in species concepts. *Bulletin du Jardin Botanique de L'Etat a Bruxelles* **37**: 31-36.

Hill, M., Witsenboer, M., Zabeau, M., Vos, P., Kesseli, R. & Michelmore, R. 1996: PCR-based fingerprinting using AFLPs as a tool for studying genetic relationships in *Lactuca* spp. *Theoretical and Applied Genetics* **93**: 1202-1210.

Hilliard, O. M. & Burtt, B. L. 1981: Some generic concepts in Compositae - Gnaphaliinae. *Botanical journal of the Linnean Society* **82**: 181-232.

Hillis, D. M. 1987: Molecular versus morphological approaches to systematics. *Annual review of ecology and systematics* **18**: 23-42.

Hoelzel, A. R. & Green, A. 1998: PCR protocols and population analysis by direct DNA sequencing and PCR-based DNA fingerprinting. *In*: Hoelzel, A. R. (ed.). Molecular Genetic Analysis of Populations, pp. 201-235. Oxford University Press, Oxford, UK.

Hombron, J. B. & Jacquinot, M. H. 1843-1853: Atlas. Voyage au Pole Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zélée, exécuté par ordre du roi pendant les années 1837-1838-1838-1840 sous le commandement de M.J. Dumont d'Urville, capitaine de vaisseau (Dumont d'Urville, J. S. C., ed.). Gide et J. Baudry, Paris.

Hombron, J. B. & Jacquinot, M. H. 1853: Botanique. *In*: Dumont d'Urville, J. S. C. (ed.). Voyage au Pole Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zélée, exécuté par ordre du roi pendant les années 1837-1838-1838-1840 sous le commandement de M.J. Dumont d'Urville, capitaine de vaisseau, Vol. 2, Plantes vasculaires, par J. Decaisne. Vol. 2. Gide et J. Baudry, Paris.

Hongtrakul, V., Huestis, G. M. & Knapp, S. J. 1997: Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. *Theoretical and Applied Genetics* **95**: 400-407.

Hooker, J. D. 1847: The botany of the antarctic voyage of H.M. discovery ships *Erebus* and *Terror*, in the years 1839-1843, under the command of Captain Sir James Clark Ross. Vol. I. Flora Antarctica pt 1. Reeve, London.

Hooker, J. D. 1852: The botany of the antarctic voyage of H.M. discovery ships *Erebus* and *Terror*, in the years 1839-1843, under the command of Captain Sir James Clark Ross. Vol. II. Flora Novae-Zealandiae pt 1. Reeve, London.

Hooker, J. D. 1855: The botany of the antarctic voyage of H.M. discovery ships *Erebus* and *Terror*, in the years 1839-1843, under the command of Captain Sir James Clark Ross. Vol. II. Flora Novae-Zealandiae pt 2. Reeve, London.

Hooker, J. D. 1860: The botany of the antarctic voyage of H.M. discovery ships *Erebus* and *Terror*, in the years 1839-1843, under the command of Captain Sir James Clark Ross. Vol. III. Flora Tasmaniae pt 1. Reeve, London.

Hooker, J. D. 1864: Handbook of New Zealand flora. Reeve, London.

Hoy, J. M. 1962: Eriococcidae (Homoptera: Coccoidae) of New Zealand. *New Zealand Department of Scientific and Industrial Research bulletin* **146**: 218 pp.

Hudson, G. V. 1928: The butterflies and moths of New Zealand. Ferguson & Osborne Ltd., Wellington.

Hudson, G. V. 1934: New Zealand beetles and their larvae. An elementary introduction to the study of our native Coleoptera. Ferguson & Osborne Ltd., Wellington.

Hudson, G. V. 1939: A supplement to the butterflies and moths of New Zealand. Ferguson & Osborne Ltd., Wellington.

Hughes, S. J. 1981: New Zealand fungi. 28. Capnodiaceae. *In*: Bilgrami, K. S., Misra, R. S. & Misra, P. C. (eds.). Advancing frontiers of mycology and plant pathology, pp. 29-36. Today & Tomorrow's Printers and Publishers, New Delhi.

Humphries, C. J. 1989: Modern data sets for flowering plants. *In*: Fernholm, B., Bremer, K. & Jörnfall, H. (eds.). The hierarchy of life. Molecules and morphology in phylogenetic analysis. Proceedings from Nobel Symposium 70 held at Alfred Nobel's Björkborn, Karlskoga, Sweden, August 29 - September 2, 1988, pp. 215-226. Elsevier Science Publishers B.V., Amsterdam.

Huys, G., Coopman, R., Janssen, P. & Kersters, K. 1996: High-resolution genotypic analysis of the genus *Aeromonas* by AFLP fingerprinting. *International journal of systematic bacteriology* **46**: 572-580.

Huys, G. & Swings, J. 1999: Evaluation of a fluorescent amplified fragment length polymorphism (FAFLP) methodology for the genotypic discrimination of *Aeromonas* taxa. *FEMS Microbiology letters* **177**: 83-92.

Janssen, P., Coopman, R., Huys, G., Swings, J., Bleeker, M., Vos, P., Zabeau, M. & Kersters, K. 1969: Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. *Microbiology* **142**: 1881-1893.

Jardine, N. 1969: A logical basis for biological classifications. *Systematic Zoology* **18**: 37-52.

Jensen, R. J. & Eshbaugh, W. H. 1976a: Numerical taxonomic studies of hybridization in *Quercus*. I. Populations of restricted areal distribution and low taxonomic diversity. *Systematic Botany* **1**: 1-10.

Jensen, R. J. & Eshbaugh, W. H. 1976b: Numerical taxonomic studies of hybridization in *Quercus*. II. Populations with wide areal distribution and high taxonomic diversity. *Systematic Botany* **1**: 11-19.

Johns, P. M. 1975: Craneflies. *New Zealand's Nature Heritage* **5**: 1867-1868.

Johnson, M. P. & Holm, R. W. 1968: Numerical taxonomic studies in the genus *Sarcostemma* R.Br. (Asclepiadaceae). *In*: Heywood, V. H. (ed.). Modern methods in plant taxonomy, pp. 199-217. Academic Press, London.

Jones, S. B. & Luchsinger, A. E. 1986: Plant Systematics. 2nd edit. McGraw-Hill Book Company, New York.

Judd, W. S., Campbell, C. S., Kellogg, E. A. & Stevens, P. F. 1999: Plant Systematics a phylogenetic approach. Sinauer Associates, Inc., Massachusetts.

Kadereit, J. W. 1994: Molecules and morphology, phylogenetics and genetics. *Botanica Acta* **107**: 369-373.

Kalin, M. 1967a: A taxonomic study of geographic variation and hybridization in populations of two *Cassinia* species. Unpublished study, University of Canterbury, New Zealand.

Kalin, M. 1967b: *Cassinia* Report. Unpublished study, University of Canterbury, New Zealand.

Kardolus, J. P., van Eck, H. J. & van den Berg, R. G. 1998: The potential of AFLPs in biosystematics: a first application in solanum taxonomy (Solanaceae). *Plant systematics and evolution* **210**: 87-103.

Karp, A. & Edwards, K. J. 1995: Molecular techniques in the analysis of the extent and distribution of genetic diversity. *In*: Ayad, W. G., Hodgkin, T., Jaradat, A. & Rao, V. R. (eds.). Molecular Genetic Techniques for Plant Genetic Resources. Report of an IPGRI Workshop, 9-11 October 1995, pp. 11-22, Rome.

Kelsey, R. G., Reynolds, G. W. & Rodriguez, E. 1984: The chemistry of biologically active constituents secreted and stored in plant glandular trichomes. *In*: Rodriguez, E., Healy, P. L. & Mehta, I. (eds.). Biology and chemistry of plant trichomes, pp. 187-241. Plenum Press, New York.

Kim, S. C., Crawford, D. J., Tadesse, M., Berbee, M., Ganders, F. R., Pirseyedi, M. & Esselman, E. J. 1999: ITS sequences and phylogenetic relationships in *Bidens* and *Coreopsis* (Asteraceae). *Systematic botany* **24**: 480-493.

King, R. M. & Robinson, H. 1970: The new synantherology. *Taxon* **19**: 6-11.

Kirk, T. 1899: The student's flora of New Zealand. Government Printer, Wellington.

Kirpicznikov, M. E. & Kuprijanova, L. A. 1950: Morphological-geographical and palynological contributions to the understanding of the genera of the sub-tribe Gnaphaliinae [In Russian]. *Acta Instituti Botanici Academiae Scientiarum URSS, series 1,* **9**: 7-37.

Koopman, W. J. M., Guetta, E., van de Wiel, C. C. M., Vosman, B. & van den Berg, R. G. 1998: Phylogenetic relationships among *Lactuca* (Asteraceae) species and related genera based on ITS-1 DNA sequences. *American journal of botany* **85**: 1517-1530.

Kovach, W. L. 1998: MVSP - A Multi Variate Statistical Package for Windows 3.0 edit. Kovach Computing Services, Pentraeth, Wales, U.K.

Kovach, W. L. 1998b: MVSP Plus version 3.0 user's manual. Kovach Computing Services, Pentraeth, Wales, U.K.

Krauss, S. L. & Peakall, R. 1998: An evaluation of the AFLP fingerprinting technique for the analysis of paternity in natural populations of *Persoonia mollis* (Proteaceae). *Australian journal of botany* **46**: 533-546.

Lamb, K. P. 1960: A Check List of New Zealand Plant Galls (Zoocecidia). *Transactions of the New Zealand Institute* **88**: 121-139.

Lamboy, W. F. 1994: The accuracy of the maximum parsimony methods for phylogeny reconstruction with morphological characters. *Systematic botany* **19**: 489-505.

Law, J. R., Donini, P., R.M.D., K., Reeves, J. C. & Cooke, R. J. 1997: Statistical methods for assessing and interpreting genetic diversity. Advances in Biometrical Genetics. Proceedings of the 10th meeting of the Eucarpia Section, Biometrics in Plant Breeding, Poznan.

Lawrence, G. H. M., Buchheim, A. F. G., Daniels, G. S. & Dolezal, H., eds. 1968: Botanico-Periodicum-Huntianum. Pittsburgh: Hunt Botanical Library.

Lloyd, D. G. 1972: A revision of the New Zealand, Subantarctic, and South American species of *Cotula*, Section Leptinella. *New Zealand journal of botany* **10**: 277-372.

Loh, J. P., Kiew, R., Set, O., Gan, L. H. & Gan, Y. 2000: A study of genetic variation and relationships within the bamboo subtribe Bambusinae using amplified fragment length polymorphism. *Annals of Botany* **85**: 607-612.

Lu, J., Knox, M. R., Ambrose, M. J., Brown, J. K. M. & Ellis, T. H. L. 1996: Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. *Theoretical and Applied Genetics* **93**: 1103-1111.

Lyon, G. L., Brooks, R. R., Peterson, P. J. & Butler, G. W. 1968: Trace elements in a New Zealand serpentine flora. *Plant and soil* **29**: 225-240.

Lyon, G. L., Brooks, R. R., Peterson, P. J. & Butler, G. W. 1970: Some trace elements in plants from serpentine soil. *New Zealand journal of science* **13**: 133-139.

Lyon, G. L., Brooks, R. R., Peterson, P. J. & Butler, G. W. 1971: Calcium, magnesium and trace elements in a New Zealand serpentine flora. *Journal of ecology* **59**: 421-429.

Majer, D., Lewis, B. G. & Mithen, R. 1998: Genetic variation among field isolates of *Pyrenopeziza brassicae*. *Plant Pathology* **47**: 22-28.

Majer, D., Mithen, R., Lewis, B. G., Vos, P. & Oliver, R. P. 1996: The use of AFLP fingerprinting for the detection of genetic variation in fungi. *Mycological Research* **100**: 1107-1111.

Mallet, J. 1995: A species definition for modern synthesis. *Trends in ecology and evolution* **10**: 294-299.

Malloch, J. R. 1931: New Zealand Muscidae Acalyptratae. Part VIII-IX. *Records of the Canterbury Museum* **3**: 389-422.

Mantel, N. A. 1967: The detection of disease clustering and a generalised regression approach. *Cancer Research* **27**: 209-220.

Markos, S. & Baldwin, B. G. 2001: Higher-level relationships and major lineages of *Lessingia* (Compositae, Astereae) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. *Systematic botany* **26**: 168-183.

Martin, W. 1932: The Vegetation of Marlborough. Reprinted from "The Marlborough Express".

Maskell, W. M. 1879: On some Coccidae in New Zealand. *Transactions of the New Zealand Institute* **12**: 291-301.

Maskell, W. M. 1885: Further notes on Coccidae in New Zealand. *Transactions of the New Zealand Institute* **17**: 20-31.

Maskell, W. M. 1891: Further coccid notes; with descriptions of new species from New Zealand, Australia and Fiji. *Transactions of the New Zealand Institute* **23**: 1-36.

Matthes, M. C., Daly, A. & Edwards, K. J. 1998: Amplified fragment length polymorphism (AFLP). *In*: Karp, A., Isaac, P. C. & Ingram, D. S. (eds.). Molecular Tools for Screening Biodiversity, pp. 183-190. Chapman and Hall, London.

Matthews, B. 1979: Growing native plants. Reed, Wellington.

Maughan, P. J., Saghai Maroof, M. A. & Buss, G. R. 1996: Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theoretical and Applied Genetics* **93**: 392-401.

Mayr, E. 1940: Speciation phenomena in birds. *American naturalist* **74**: 249-278.

Mayr, E. 1969: The biological meaning of species. *Biological journal of the Linnean Society* **1**: 311-320.

McNeill, J. 1979: Structural value: a concept used in the construction of taxonomic classifications. *Taxon* **28**: 481-504.

Merxmüller, H., Leins, P. & Roessler, H. 1977: Inuleae - systematic review. *In*: Heywood, V. H., Harborne, J. B. & Turner, B. L. (eds.). The biology and chemistry of the Compositae. Academic Press, London.

Metcalf, L. J. 2000: New Zealand trees & shrubs: a comprehensive guide to cultivation and identification. Reed, Auckland.

Meyrick, E. 1936: Descriptions and notes on New Zealand Lepidoptera. *Transactions of the Royal Society of New Zealand* **66**: 281-283.

Miller, D. 1971: Common insects in New Zealand. Reed, Wellington.

Milligan, B. G. 1998: Total DNA isolation. *In*: Hoelzel, A. (ed.). Molecular genetic analysis of populations: a practical approach, pp. 29-64. Oxford University Press, New York.

Mishler, B. D. 2000: Deep phylogenetic relationships among "plants" and their implications for classification. *Taxon* **49**: 661-683.

Molloy, B. P. J. 1959: A study in subalpine plant ecology on Fog Peak Ridge, Porter's Pass, Canterbury. Unpublished thesis presented for the degree of Master of Science with Honours in Botany, University of Canterbury, New Zealand.

Molloy, B. P. J. 1995: Two new species of *Leucogenes* (Inuleae: Asteraceae) from New Zealand, and typification of *L. grandiceps*. *New Zealand journal of botany* **33**: 53-63.

Molloy, B. P. J., Burrows, C. J., Cox, J. E., Johnston, J. A. & Wardle, P. 1963: Distribution of subfossil remains, eastern South Island, New Zealand. *New Zealand journal of botany* **1**: 68-77.

Moritz, C. & Hillis, D. M. 1996: Molecular Systematics: Context and Controversies. *In*: Hillis, D. M., Moritz, C. & Mable, B. K. (eds.). Molecular Systematics, pp. 1-13. Sinauer Accociates, Inc., Massachusetts.

Mueller, U. G. & Wolfenbarger, L. L. 1999: AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution* **14**: 389-394.

Myers, J. G. 1922: The order Hemiptera in New Zealand, with species reference to its biological and economic aspects. *New Zealand journal of science and technology* **5**: 1-12.

Myers, J. G. 1923: A contribution to the study of the New Zealand leaf-hoppers and plant-hoppers (Cicadellidae and Fulgoroidae). *Transactions of the New Zealand Institute* **54**: 407-429.

Myers, J. G. 1926: Biological notes on New Zealand Heteroptera. *Transactions of the New Zealand Institute* **56**: 449-511.

Noyes, R. D. & Rieseberg, L. H. 1999: ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster* s.L. *American journal of botany* **86**: 398-412.

O'Hanlon, P. C., Peakall, R. & Briese, D. T. 1999: Amplified fragment length polymorphism (AFLP) reveals introgression in weedy *Onopordum* thistles: hybridization and invasion. *Molecular Ecology* **8**: 1239-1246.

Patterson, C. 1987: *In*: Patterson, C. (ed.). Molecules and Morphology in Evolution: Conflict or Compromise?, pp. 1-23. Cambridge University Press, Cambridge.

Patterson, C., Williams, D. M. & Humphries, C. J. 1993: Congruence between molecular and morphological phylogenies. *Annual review of ecology and systematics* **24**: 135-188.

Pearson, K. 1901: On lines and planes of closest fit to a system of points in space. *Philosophical magazine and journal of science, series 6*: 559-572.

Pennycook, S. R. 1989: Fungal plant diseases recorded in New Zealand. Plant diseases recorded in New Zealand (Division, P. D., ed.). 2. DSIR, Auckland.

Perrie, L. R., Lockhart, P. J., Brownsey, P. J. & Large, M. F. 2000: Morphological and molecular concordance for the recognition of two species in the New Zealand *Polystichum richardii* (Hook.) J. Smith complex. *Plant systematics and evolution* **224**: 97-107.

Pielou, E. C. 1984: The interpretation of ecological data. Wiley. New York.

Poole, A. L. & Adams, N. M. 1994: Trees and shrubs of New Zealand. Manaaki Whenua Press, Landcare Research, Lincoln, Canterbury, New Zealand.

Powell, W., Morgante, M., McDevitt, R., Vendramin, G. & Rafalski, A. 1995: Polymorphic simple sequence repeat regions in chloroplast genomes: Applications to the population genetics of pines. *Proceedings of the National Academy of Science USA* **92**: 7759-7763.

Primack, R. B. 1983: Insect pollination in the New Zealand mountain flora. *New Zealand journal of botany* **21**: 317-333.

Qamaruz-Zaman, F., Fay, M. F., Parker, J. S. & Chase, M. W. 1998: The use of AFLP fingerprinting in conservation genetics: a case study of *Orchis simia* (Orchidaceae). *Lindleyana* **13**: 125-133.

Raoul, E. F. L. 1846: Choix de plantes de la Nouvelle-Zélande. Fortin, Masson et Cie, Paris.

Raven, P. H. 1974: Plant systematics 1947-1972. *Annals of the Missouri Botanical Garden* **61**: 166-178.

Raven, P. H. & Raven, T. E. 1976: The genus *Epilobium* (Onagraceae) in Australasia: a systematic and evolutionary study. Botany Division, Christchurch.

Reid, A. R. & Bohm, B. A. 1994: Vacuolar and exudate flavonoids of New Zealand *Cassinia* (Asteraceae, Gnaphalieae). *Biochemical systematics and ecology* **22**: 501-505.

Reid, A. R. & Bohm, B. A. 1995: Vacuolar flavonoids of *Raoulia* (Inuleae; Gnaphaliinae; Asteraceae). *Biochemical systematics and ecology* **23**: 209.

Richard, A. 1832: Essai d'une Flore de la Nouvelle Zélande. *In*: Dumont d'Urville, J. S. C. (ed.). Voyage de decouvertes de'Astrolabe exécuté par ordre du Roi, pendant les années 1826-1827-1828-1829, sous le commandement de M.J. Dumont d'Urville. Botanique par MM. A. Lesson et A. Richard. Tastu, Paris.

Rieseberg, L. H., Kim, M. J. & Seiler, G. J. 1999: Introgression between the cultivated sunflower and a sympatric wild relative, *Helianthus petiolaris* (Asteraceae). *International journal of plant science* **160**: 102-108.

Rieseberg, L. H., Whitton, J. & Linder, C. R. 1996: Molecular marker incongruence in plant hybrid zones and phylogenetic trees. *Acta Botanica Neerlandica* **45**: 243-262.

Roa, A. C., Maya, M. M., Duque, M. C., Tohme , J., Allem, A. C. & Bonierbale, M. W. 1997: AFLP analysis of relationships among cassava and other *Manihot* species. *Theoretical and Applied Genetics* **95**: 741-750.

Rohlf, F. J. 1997: NTSYS-pc: Numerical Taxonomy and Multivariate System, version 2.01h. Exeter Software, Setauket, New York.

Rohlf, F. L. 1963: Congruence of larval and adult classification in *Aedes* (Diptera: Culicidae). *Systematic Zoology* **12**: 97-117.

Roldán-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A. & De Loose, M. 2000: AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding* **6**: 125-134.

Roldán-Ruiz, I., Depicker, A., Dendauw, J., Van Bockstaele, E. & De Loose, M. 1998: Comparison of different methods to score radioactive AFLP gels. Proceedings of the 6th Plant and Animal Genome Meeting, San Diego.

Rosendahl, S. & Taylor, J. W. 1997: Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP. *Molecular Ecology* **6**: 821-829.

Roy, B. 1998: An illustrated guide to common weeds of New Zealand. New Zealand Plant Protection Society, Lincoln, New Zealand.

Russel, J. R., Weber, J. C., Booth, A., Powell, W., Sotelo-Montes, C. & Dawson, I. K. 1999: Genetic variation of *Calycophyllum spruceanum* in the Peruvian Amazon Basin, revealed by amplified fragment length polymorphism (AFLP) analysis. *Molecular Ecology* **8**: 199-204.

Salmon, J. T. 1991: Native New Zealand flowering plants. Reed, Auckland.

Salmon, J. T. 1992: A field gued to the alpine plants of New Zealand. 3rd edit. Godwit Publishing Ltd, Auckland.

Schaal, B. A., Hayworth, D. A., Olsen, K. M., Rauscher, J. T. & Smith, W. A. 1998: Phylogeographic studies in plants: problems and prospects. *Molecular Ecology* **7**: 465-474.

Schilling, E. E. J. & Heiser, C. B. J. 1976: Re-examination of a numerical taxonomic study of *Solanum* species and hybrids. *Taxon* **25**: 451-462.

Schlötterer, C. 1998: Microsatellites. *In*: Hoelzel, A. R. (ed.). Molecular Genetic Analysis of Populations, pp. 237-261. Oxford Univerity Press, Oxford, UK.

Scott, R. R., ed. 1984: New Zealand pest and beneficial insects. Christchurch: Lincoln University College of Agriculture.

Segarra, J. G. & Mateu, I. 2001: Taxonomic study of *Linaria depauperata* and *L. supina* complexes in eastern Spain. *Annals of Botany* **87**: 157-177.

Semblat, J. P., Wajnberg, E., Dalmasso, A., Abad, P. & Castagnone-Sereno, P. 1998: High-resolution DNA fingerprinting of parthenogenic root-knot nematodes using AFLP analysis. *Molecular Ecology* **7**: 119-125.

Sheppard, J. S. 1965: The role of *Cassinia fulvida* R. Br. in the succession of scrub at Cass, Canterbury. Unpublished thesis presented for the degree of Master of Science with Honours in Botany, University of Canterbury, New Zealand.

Skvarla, J. J., Turner, B. L., Patel, V. C. & Tomb, A. S. 1977: Pollen morphology in the Compositae and in morphologically related families. *In*: Heywood, V. H., Harborne, J. B. & Turner, B. L. (eds.). The Biology and Chemistry of the Compositae, pp. 141-265. Academic Press, London.

Smith, J. J., Scott-Craig, J. S., Leadbetter, J. R., Bush, G. L., Roberts, D. L. & Fulbright, D. W. 1994: Characterization of random amplified polymorphic DNA (RAPD) products from *Xanthomonas campestris* and some comments on the use of RAPD products in phylogenetic analysis. *Molecular Phylogenetic Evolution* **3**: 135-145.

Sneath, P. H. A. 1957: The application of computers to taxonomy. *J. Gen. Microbiol.* **17**: 201-226.

Sneath, P. H. A. & Sokal, R. R. 1973: Numerical taxonomy. The principles and practice of numerical classification. Freeman and Company, San Francisco.

Sokal, R. R. 1973: The species problem reconsidered. *Systematic zoology* **22**: 360-374.

Sokal, R. R. 1979: Testing statistical significance of geographic variation patterns. *Systematic Zoology* **28**: 227-232.

Sokal, R. R. & Rohlf, F. J. 1962: The comparison of dendrograms by objective methods. *Taxon* **11**: 33-40.

Sokal, R. R. & Sneath, P. H. A. 1963: Principles of Numerical Taxonomy. W.H. Freeman and Company, San Francisco.

Soltis, P. S., Soltis, D. E. & Chase, M. W. 1999: Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* **402**: 402-404.

Soltis, P. S., Soltis, D. E. & Doyle, J. J. 1992: Molecular Systematics of Plants. Chapman and Hall, New York.

Spiller, D. M. & Wise, K. A. J., eds. 1982: A catalogue (1860-1960) of New Zealand insects and their host plants compiled by D.M. Spiller and K.A.J. Wise. DSIR Bulletin 231. Edited by Dale, P. S. & Maddison, P. A. Wellington: G. P. Sutherland, Science Information Division, DSIR.

Spooner, D. M., Van den Berg, R. G. & J.T., M. 2000: Species and series boundaries of *Solanum* series *Longipedicellata* (Solanaceae) and phenetically similar species in ser. *Demissa* and ser. *Tuberosa*: Implications for a practical taxonomy of section *Petota*. *American journal of botany* **87**: 113-130.

Stace, C. A. 1989: Plant taxonomy and biosystematics. 2nd edit. Edward Arnold, New York.

Stafleu, F. A. & Cowan, R. S. 1976: Taxonomic Literature. 2nd edit. Vol. 1: A-G. Bohn, Scheltema and Holkema, Utrecht.

Stafleu, F. A. & Cowan, R. S. 1979: Taxonomic Literature. Vol. 2: H-Le. Bohn, Scheltema and Holkema; Junk, Utrecht.

Stafleu, F. A. & Cowan, R. S. 1983: Taxonomic Literature. 2nd edit. Vol. 4: P-Sak. Bohn, Scheltema and Holkema; Junk, Utrecht.

Statistical Sciences 1998: S-Plus Version 4.5 Professional Release 2. MathSoft, Inc., Seattle, Washington.

Stearn, W. T. 1992: Botanical Latin: history, grammar, syntax, terminology and vocabulary. 4th edit. Newton Abbott: David & Charles, Edinburgh.

Stebbins, G. L. 1950: Variation and evolution in plants. Columbia University Press, New York.

Stuessy, T. F. 1972: Revision of the genus *Melampodium* (Compositae: Heliantheae). *Rhodora* **74**: 1-70, 161-219.

Stuessy, T. F. 1990: Plant taxonomy: The systematic evaluation of comparative data. Columbia University Press, New York.

Sykes, W. R. 1992: Two new names in *Macropiper* Miq. (Piperaceae) from New Zealand. *New Zealand journal of botany* **30**: 231-236.

Tanksley, S. D., Young, N. D., Paterson, A. H. & Bonierbale, M. W. 1989: RFLP marking in plant breeding: new tools for an old science. *Biotechnology* **7**: 257-264.

Thiele, K. 1993: The holy grail of the perfect character: the cladistic treatment of morphometric data. *Cladistics* **9**: 275-304.

Thorne, R. F. 2000: The classification and geography of the flowering plants: dicotyledons of the class Angiospermae. *The botanical review* **66**: 441-647.

Thorpe, R. S. 1983: A review of the numerical methods for recognising and analysing racial differentiation. *In*: Felsenstein, J. (ed.). Numerical taxonomy. Springer-Verlag, Berlin.

Tillyard, R. J. 1926: The insects of Australia and New Zealand. Angus & Robertson, Sydney.

Tohme, J., Gonzalez, D. O., Beebe, S. & Duque, M. C. 1996: AFLP analysis of gene pools of a wild bean core collection. *Crop Science* **36**: 1375-1384.

Travers, W. T. L. 1874: On the spread of *Cassinia leptophylla*, Art. XLIII. *Transactions of the New Zealand Institute* **VI**: 248-251.

Travis, S. E., Maschinski, I. & Keim, P. 1996: An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. *Molecular Ecology* **5**: 735-745.

Triest, L. 2001: Hybridization in staminate and pistillate *Salix alba* and *S. fragilis* (Salicaceae): morphology versus RAPDs. *Plant systematics and evolution* **226**: 143-154.

Urbatsch, L. E., Baldwin, B. G. & Donoghue, M. J. 2000: Phylogeny of the coneflowers and relatives (Heliantheae : Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chlorplast DNA restriction site data. *Systematic botany* **25**: 539-565.

Van Huylenbroeck, J. M., De Riek, J. & De Loose, M. 2000: Genetic relationships among *Hibiscus syriacus*, *Hibiscus sinosyriacus* and *Hibiscus paramutabilis* revealed by AFLP, morphology and ploidy analysis. *Genetic resources and crop evolution* **47**: 335-343.

Vaz Patto, M. C., Aardse, A., Buntjier, J. & Rubiales, D. 2001: Morphology and AFLP markers suggest three *Hordeum chilense* ecotypes that differ in avoidance to rust fungi. *Canadian journal of botany* **79**: 204-213.

Van Valen, L. 1976: Ecological species, multispecies, and oaks. *Taxon* **25**: 233-239.

Vijverberg, K. 2001: Adaptive radiation of Australian and New Zealand *Microseris* (Asteraceae) a case study based on molecular and morphological markers. University of Amsterdam.

Vos, P. R., Hogers, M., Bleeker, M., van de Lee, T., Hornes, M., Frijters, A., Peleman, J., Kuiper, M. & Zabeau, M. 1995: AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407-4414.

Ward, J. M. 1981: Numerical phenetics and the classification of *Raoulia* (Gnaphaliinae - Compositae). Ph.D. Thesis, University of Canterbury, Christchurch, New Zealand.

Ward, J. M. 1993a: Systematics of the New Zealand Inuleae (Compositae - Asteraceae) 1: A numerical phenetic study of the species of *Raoulia*. *New Zealand journal of botany* **31**: 21-28.

Ward, J. M. 1993b: Systematics of the New Zealand Inuleae (Compositae - Asteraceae) 2: A numerical phenetic study of *Raoulia* in relation to allied genera. *New Zealand journal of botany* **31**: 29-42.

Ward, J. M. & Breitwieser, I. 1998: Systematics of the New Zealand Inuleae (Compositae) - 4. A taxonomic review. *New Zealand journal of botany*.

Ward, J. M., Breitwieser, I. & Lovis, J. 1997: *Rachelia glaria* (Compositae), a new genus and species from the South Island, New Zealand. *New Zealand journal of botany* **35**: 145-154.

Wardle, P. 1963: Growth habits of New Zealand subalpine shrubs and trees. *New Zealand journal of botany* **1**: 18-47.

Wardle, P. 1991: Vegetation of New Zealand. Cambridge University Press, Cambridge.

Waugh, R., Bonar, N., Baird, E., Thomas, B., Graner, A., Hayes, P. & Powell, W. 1997: Homology of AFLP products in three mapping populations of barley. *Molecular and General Genetics* **255**: 311-321.

Webb, C. J. 1987: *Anaphalis*. In: Connor, H.E.; Edgar, E. Name changes in the indigenous New Zealand flora, 1960-1986 and Nomina Nova IV, 1983-1986. *New Zealand journal of botany* **25**: 147.

Webb, C. J. 1988: Asteraceae: Inulae. *In*: Webb, C., Sykes, W. R. & Garnock-Jones, P. J. (eds.). Flora of New Zealand. Vol. IV. Botany Division, Department of Scientific and Industrial Research, Christchurch, New Zealand.

Webb, C. J., Sykes, W. R. & Garnock-Jones, P. J. 1988: Flora of New Zealand. Vol. IV. Botany Division, Department of Scientific and Industrial Research, Christchurch, New Zealand.

Whiffin, T. 1973: Analysis of a hybrid swarm between *Heterocentron elegans* and *H. glandulosum* (Melastomataceae). *Taxon* **22**: 413-423.

Williams, C. E. & St. Clair, D. A. 1993: Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accession of *Lycopersicon esculentum*. *Genome* **36**: 619-630.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531-6535.

Wilson, H. D. 1996: Wild Plants of Mount Cook National Park. Manuka Press, Christchurch.

Wilson, H. D. & Galloway, T. 1993: Small-leaved shrubs of New Zealand. Manuka Press, Christchurch.

Wilton, A. D. 1997: An evolutionary investigation on the New Zealand Inuleae (Compositae): Stem anatomy and flowering phenology. Ph.D. Thesis, University of Canterbury, Christchurch, New Zealand.

Wilton, A. D. 1999: Phenetic Library for S-Plus 4.5 (r2). Unpublished computer routines, 23 March 1999. Landcare Research, Lincoln.

Winfield, M. O., Arnold, G. M., Cooper, F., LeRay, M., White, J., Karp, A. & Edwards, K. J. 1998: A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the upper Severn area of the UK using AFLP markers. *Molecular Ecology* **7**: 3-10.

Wolfe, A. D. & Liston, A. 1998: Contributions of PCR-based methods to plant systematics and evolutionary biology. *In*: Soltis, D. E., Soltis, P. S. & Doyle, J. J. (eds.). Molecular systematics of plants. Vol. 2, pp. 43-86. Kluwer Academic Publishers, Dordrecht.

Wollenweber, E. 1984: The systematics and implications of flavonoids secreted by plants. *In*: Rodriguez, E., Healey, P. L. & Mehta, I. (eds.). Biology and chemistry of plant trichomes, pp. 53-69. Plenum Press, New York.

Wong, K. C. & Sun, M. 1999: Reproductive biology and conservation genetics of *Goodyera procera* (Orchidaceae). *American journal of botany* **86**: 1406-1413.

Wood, J. E. 1992: A study on a New Zealand native plant *Cassinia fulvida*. Unpublished thesis submitted in partial fulfilment of requirements for the degree of Master of Science in Chemistry, University of Canterbury.

Wood, J. E., Munro, M. H. G., Blunt, J. W. & Perry, N. B. 1999: Biologically active compounds from *Ozothamnus leptophyllus*. *New Zealand journal of botany* **37**: 167-174.

Appendix 1

Table 1 Classification of *Ozothamnus leptophyllus* in taxonomic literature.

Literature	Classification of *Ozothamnus leptophyllus*														
Breitwieser & Ward (1997)	*Ozothamnus leptophyllus* (G.Forst.) Breitw. et J.M. Ward														
Webb (1988)															
Allan (1961)	*C. retorta* A.Cunn. ex DC.	*C. leptophylla* (G.Forst.) R.Br		*C. amoena* Cheeseman	*C. fulvida* Hook.f.	*C. fulvida* var. *montana* Allan	*C. leptophylla* var. *spathulata* (Colenso) Kirk	*C. vauvilliersii* (Hombr. et Jacq.) Hook.f.	*C. vauvilliersii* var. *albida* Kirk	*C. vauvilliersii* var. *canescens* (Cockayne) Allan	*C. vauvilliersii* var. *rubra* (Buchanan) Cheeseman (plant with an "uncertain status")	*C. vauvilliersii* var. *serpentina* Cockayne et Allan	*C. vauvilliersii* var. *pallida* Allan	Hybrid	Hybrid
Carse (1930)														x *C. amoenatorta* (*C. amoena* x *retorta*)	
Cockayne & Allan (1926)												*C. vauvilliersii* var. *serpentina* Cockayne et Allan			
Cheeseman (1925)	*C. retorta* A.Cunn. ex DC.	*C. leptophylla* R.Br.		*C. amoena* Cheeseman	*C. fulvida* Hook.f.			*C. vauvilliersii* Hook.f.	*C. vauvilliersii* var. *albida* Kirk		*C. vauvilliersii* var. *rubra* (Buchanan) Cheeseman				*C. fulvida* var. *linearis* Kirk
Cheeseman (1914)	*C. retorta* A.Cunn. ex DC.	*C. leptophylla* R.Br.		*C. amoena* Cheeseman	*C. fulvida* Hook.f.			*C. vauvilliersii* Hook.f.	*C. albida* (Kirk) Cockayne						
Cockayne (1906)									*C. albida*	*C. albida* var. *canescens*					
Cheeseman (1906)	*C. retorta* A.Cunn. ex DC.	*C. leptophylla* R.Br		*C. amoena* Cheeseman	*C. fulvida* Hook.f.			*C. vauvilliersii* Hook.f.	*C. vauvilliersii* var. *albida* Kirk		*C. vauvilliersii* var. *rubra* (Buchanan) Cheeseman				*C. fulvida* var. *linearis* Kirk
Kirk (1899)	*C. retorta* A.Cunn. ex DC.	*C. leptophylla* R.Br		*C. amoena* Cheeseman	*C. fulvida* Hook.f.		*C. leptophylla* var. *spathulata* (Colenso) Kirk	*C. vauvilliersii* Hook.f.	*C. vauvilliersii* var. *albida*		*C. rubra* Buchanan				*C. fulvida* var. *linearis*
Cheeseman (1897)				*C. amoena* n. sp.											
Colenso (1890)							*C. spathulata*								
Colenso (1888)								*Olearia xanthophylla*							
Buchanan (1887)											*C. rubra* Buchanan				
Hooker (1864)	*C. retorta* A.Cunn.	*C. leptophylla* R.Br			*C. fulvida* Hook.f.			*C. vauvilliersii* Hook.f.							
Hooker (1853)	*C. retorta* A.Cunn.	*C. leptophylla* R.Br.	*C. leptophylla* var. β Hook.f.		*C. leptophylla* var. γ Hook.f.			*C. vauvilliersii* Hook. f.							
Hooker (1847)								*Ozothamnus vauvilliersii* Hombr. et Jacq.							

Table 1 continued:

Literature	Classification of *Ozothamnus leptophyllus*														
Hombron & Jacquinot (1853)								*Ozothamnus vauvilliersii* Hombr. et Jacq.							
Raoul (1844)	*C. retorta* A.Cunn.	*C. leptophylla* R.Br.													
Cunningham (1839)	*C. leptophylla* A.Cunn non *Calea leptophylla* G.Forst														
Cunningham ex Candolle (1837)	*C. retorta* A. Cunn.	*Cassinia. leptophylla* R.Br.													
Richard (1832)		*Calea leptophylla* G.Forst													
Brown (1817)		*Cassinia leptophylla*													
Forster (1786)		*Calea leptophylla*													

Appendix 1

Table 2 Potential type material in *Cassinia*.

Allan 1961	Herbarium specimens with all informations given on herbarium sheet
C. amoena Cheeseman	**AK: 10298** Coll.: T.F. Cheeseman Jan. 1896, North Cape. Herbarium T.F. Cheeseman Remark on label: Illustrations of the New Zealand Flora, Plate 107 (Cheeseman 1914) **AK: 10299** Coll.: T.F. Cheeseman Jan. 1896, North Cape. Herbarium T.F. Cheeseman **WELT: 58304** Coll.: T.F. Cheeseman Jan. 1896, North Cape. *C. amoena* underlined in red ink with a note: "not yet examined, but has the aspect of C. vauvilliersii North Cape, extreme abundant" Herbarium T. Kirk ex Herb. T.F. Cheeseman
C. fulvida Hook.f.	**K**: Coll.: Lyall (right-hand top corner specimen), no locality, no date; 5 other specimens on herbarium sheet, 3 are labelled: Coll.: Hector & Buchanan, Otago 1863; Coll.: ?, Lake District; No. 28, common over Otago, Flowers all summer; Coll.: ?, Hurunui Haven 1864. Herbarium Hookerianum 1867
C. fulvida var. linearis Kirk	**WELT: 58665** Coll.: Bernard Cracroft Aston, 02.02.1896, Southern Reservoir, Dunedin, (Lat. 45°54'- S; Long. 170°27' -E) D. Petrie Herbarium **WELT: 58747/A** Coll.: Bernard Cracroft Aston, 02.02.1896, Southern Reservoir, Dunedin, (Lat. 45°54'- S; Long. 170°27' -E) T. Kirk Private Herbarium **WELT: 58747/B** Coll.: Bernard Cracroft Aston, 02.02.1896, Southern Reservoir, Dunedin, (Lat. 45°54'- S; Long. 170°27' -E) T. Kirk Private Herbarium **WELT: 58748/A** Coll.: Bernard Cracroft Aston, 02.02.1896, Southern Reservoir, Dunedin, (Lat. 45°54'- S; Long. 170°27' -E) T. Kirk Private Herbarium **WELT: 58748/B** Coll.: Bernard Cracroft Aston, 02.02.1896, Southern Reservoir, Dunedin, (Lat. 45°54'- S; Long. 170°27' -E) T. Kirk Private Herbarium **WELT: 58749** Coll.: Bernard Cracroft Aston, 02.02.1896, Southern Reservoir, Dunedin, (Lat. 45°54'- S; Long. 170°27' -E) T. Kirk Private Herbarium **WELT: 58749** Coll.: Bernard Cracroft Aston, 02.02.1896, Southern Reservoir, Dunedin, (Lat. 45°54'- S; Long. 170°27' -E) T. Kirk Private Herbarium
C. fulvida var. *montana* Allan	**CHR: 9397** Coll.: V.D. Zotov, 15.01.1936, Arthur's Pass, Canterbury, Alt. 914 m Remarks: achenes pubescent, bract pubescent, rays numerous

Table 2 continued

Allan 1961	Herbarium specimens with all informations given on herbarium sheet
C. leptophylla (G.Forst.) R.Br.	**K**: Coll.: G. Forster, no date, labelled as "*Gnaphalium leptophyllum*, Habitat in New Zeeland" (Forster's handwriting); 3 specimens on herbarium sheet; one fruiting. Remarks F.R. Fosberg 1990: should be moved to *Cassinia leptophylla* folder when there is room for it; *Cassinia lepthophylla* (Forst. F.); This is clearly the sheet that Allan, Fl.N.Z.1: 727, 1961 indicated as Type of *Calea leptophylla* Forst. F. The Forster Herbarium. Presented by the Corporation of Liverpool, August, 1885 **BM: 000602390 Type**: *Calea cinerea*; Coll.: Banks & Solander 1769, *Calea cinerea*, *Calea leptophylla*, Forst. Prodr. F. 55, *Cassinia leptophylla* R.Br. in Trans. Linn. Soc. Lond. 12, 1817, 126 *New Zealand 1769-70 Banks & Solander, Solander Prim.Fl.N.Zel. (Primitiae Florae Novae Zealandiae, never published) p. 519, Parkinson Ic. 100 (98??)* at the back of the sheet: Nova Zelandia, in campus nemoris prope Teoneroa (Poverty Bay, 8-11 October 1769), Tolega (-Bay, 23-29 October 1769), Opoorage (Mercury Bay, 5-15 November 1769), Motu aro (Island, 29 November and 2 December 1769), Totara nui (Queen Charlotte Sound, 15 January-6 February 1770)- Sir J. Banks & Dr. Solander **BM: 000602391**: Coll.: G. Forster?, no date, no locality, *Calea leptophylla*, *Cassinia leptophylla* Brown; 174. 289. *Calea leptophylla*; G. Forsters Herbarium (written on back of sheet) **BM: 000602392:** Coll.: G. Forster?, no date, no locality, *Calea leptophylla* Lin: Wild, *Cassinia leptophylla* Brown; Herbarium Pallas (Forsters Herb: (written on back of sheet))
C. leptophylla var. *spathulata* (Colenso) Kirk	**WELT: 24140** Coll.: William Colenso, 3 labels: A label in the hand of VD Zotov reads: "Only spn. VDZ ?". A label in the hand of W. Colenso reads: "*Cassinia spathulata*, Col.". The T.F. Cheeseman label reads: "*Cassinia leptophylla* Br. Hawkes Bay. Type of *C. spathulata*, Col." W. Colenso Herbarium **WELT: 58873** Coll. William Colenso, Napier T. Kirk Herbarium
C. retorta A. Cunn. *ex DC.*	**K**: Coll.: R. Cunningham, 1834, Hokianga River (No. 64); underneath this label: No 447 (*Cassinia leptophylla* ? R.Br.) 2. *Cassinia retorta* A. Cunn Prod VI. 155 Fl. Zeal 1132 New Zealand A. Cunningham 1826 R. Cunningham 1834. 2 specimens of *C. leptophylla* on the herbarium sheet: No. 49 ?-Bay Waikato inlet (9/64); No. 8? Port Hills Nelson (8/64). Allan Cunningham's New Zealand Herbarium. Presented by Robert Heward Esq. 1862

Table 2 continued

Allan 1961	Herbarium specimens with all informations given on herbarium sheet
C. vauvilliersii (Hombr. et Jacq.) Hook.f.	**P: 00179961** Coll.: Jacques Bernard Hombron, Voyage de l'Astrolabe et de la Zélée 1838-1840. Iles Auckland-Sud de la Nouvelle Zélande. Note under species name: Voy. au pole Sud, Pl. 5 Phan. ("first description" is plate 5 in Bot. Dicot. Phanerog.) Description was done 1841 **BM: 000602396**, ~~*Craspedia*~~ *Cassinia vauvilliersii* Hook. F., Lake Fuym??, N. Zealand, 3000f, Coll.: Hector 1826? **BM: 000602395**, Antarctic Expedition 1901-4, Auckland Island **BM: 000602394**, *Ozothamnus vauvilliersii* Hombr. & Jacq. Lord Auckland' group, *Ozothamnus vauvilliersii* H. & Jacq., Lord Auckland's Island, Antarct. Exp. 1839 - 1843. J.D.H., Coll.: Hooker. 3 specimens on one sheet: Cassinia Hook. Fl. Nov. Zel. I. 133; *Ozothamnus vauvilliersii*, Hombr. & Jacq. - Hook. fil. Fl. Antarct. p.29; on the back of the sheet: Antarctic Region Dr. J.D. Hooker **BM: 000602393**, Coll.: Forster?. No date, no locality; *Cassinia leptophylla*, R. Br. In Linn. Trans. 12, p. 126; Calea; Note on sheet: "This is wrong, cf. *Ozothamnus vauvilliersii* and *O. cenereus*". At the back of the sheet: Dusky Bay? Nova Zelandia 'Ref' Forsters **BM: 000602397**, Coll.: J.D. Hooker. Antarctic Expedition. *Ozothamnus vauvilliersii* Hombr. & Jacq. Lord Auckland's group; *Cassinia vauvilliersii* Hook.f. New Zealand: Auckland Islands (1839-1843). **BM: 000602399**, Coll.: R.M. Cormick, Bequeathed 1890. Date: 1840, H.M.S. Erebus. Ross' Antarctic Exped.; Flora of Auckland Islands; Cassinia vauvilliersii (Homb. & Jacq.). **BM: 000602398**, Coll.: R.M. Cormick, Bequeathed 1890. Date: 01.12.1840, Locality: Sandy Bay; H.M.S. Erebus. Ross' Antarctic Exped.; Flora of Auckland Islands; Cassinia vauvilliersii (Homb. & Jacq.)Hook.f. **BM: 000602400**, Coll.: R.M. Cormick, Bequeathed 1890. Date: 29.11.1840, Locality: Auckland Island "Point", H.M.S. Erebus. Ross' Antarctic Exped.; Flora of Auckland Islands; *Cassinia vauvilliersii* (Homb. & Jacq.)Hook.f.
C. vauvilliersii var. *albida*	**WELT: 58218** Coll.: Thomas Kirk, 14.12.1889, Mt. Fyffe, Kaikouras, (Lat. 42°19'- S; Long. 173°37' -E), Alt. 923 m (3000 ft). "albida" underlined in red ink; T. Kirk Herbarium **WELT: 29634** Coll.: Thomas Kirk, Mt. Fyffe, Kaikouras, Inland (Lat. 42°19'- S; Long. 173°37' -E); Kirk has written on label: "I am disposed to refer this to *C. fulvida* notwithstanding it looks so unlike that plant. It is of very robust habit, extremely glutinous, and with very large leaves. The flowers also are large but there is no structural difference." T. Kirk Herbarium **WELT: 58314** mixed collection from Kirk's Herbarium and labelled by him. Two upper pieces probably collected by John Buchanan at "West Cape" and listed in the original description of *Cassinia vauvilliersii* var. *albida* (Kirk 1899). They can be referred to a label in Kirk's hand that reads: "*Cassinia vauvilliersii*, Hook.f. var. *albida*. West Cape. Col[onial] Mus[eum]." Lower two specimens: Coll.: J. Rutland, Kaikouras. **AK: 10304** Coll.: Thomas Kirk, Kaikoura Mountains (Lat. 42°24'- S; Long. 173°39' -E); Herbarium T.F. Cheeseman **AK: 30904** Coll.: Thomas Kirk, Kaikoura Mountains (Lat. 42°24'- S; Long. 173°39' -E); Herbarium T. Kirk

Table 2 continued

Allan 1961	Herbarium specimens with all informations given on herbarium sheet
C. vauvilliersii var. *canescens* (Cockayne) Allan	**WELT: 58279** Coll.: Leonard Cockayne, 10.02.1892, Mt. Fyffe, Marlborough, South Island of New Zealand (Lat. 42°19'- S; Long. 173°37' -E), Alt. 2000 ft. Label reads: " *Cassinia* sp. new form to me but possibly *C. vauvilliersii*, if so very different to plants I have so named. Forming greater part of subalpine vegetation on Mt. Fyffe. 3 specimens on sheet. D. Petrie has attached a label to each specimen; each label reads "Mt. Fyffe". D. Petri Herbarium; ex L. Cockayne Herbarium No. 39 **WELT: 58358** Coll.: Leonard Cockayne, 07.10.1905, Mt. Fyffe (Lat. 42°19'- S; Long. 173°35' -E), Alt. 900 m. Label reads: "Type, Cassinia albida (T. Kirk) Cockayne var. canescens Cockayne, subalpine ? Mt. Fyffe at about 900 m. Coll: L.C. 7/Oct/1905, as sent to Man. ed. 2" L. Cockayne Herbarium No.9093
C. vauvilliersii var. *pallida* Allan	**CHR: 87404** Coll.: H.H. Allan, 21.03.1951, Robinson Creek, Upper Awatere, Alt. 1372 m
C. vauvilliersii var. *serpentina* Cockayne et Allan	**AK: 31977** Coll.: Leonard Cockayne, Dec. 1916, Nelson, Dun Mountain, mineral belts, (Lat. 41°21'- S; Long. 173°22' -E) Herbarium Leonard Cockayne: 1930
x *C. amoenatorta (C. amoena* x *retorta)* Carse	**CHR: 333803**, Coll.: H. Carse, Dec. 1926, Northland, Near North Cape, (Lat. 34°27'- S; Long. 172°59' -E) The Carse Herbarium, no. 1589a/5 **CHR: 333805**, Coll.: H. Carse, Dec. 1926, Northland, Near North Cape, (Lat. 34°27'- S; Long. 172°59' -E) The Carse Herbarium, no. 1589a

Appendix 2

Parasitic insects found on *Ozothamnus leptophyllus*.

Table 1 Parasites recorded on '*Cassinia* spp.'

species	order and family	literature	remarks (common name, distribution, etc.)
Bipunctoris cassinianus Eyles et Carvalho	Hemiptera: Miridae	Eyles and Carvalho 1995	North-east of the South Island
Eriococcus parabilis Hoy	Hemiptera: Coccidae	Hoy 1962	Scale Insect, Desert Road
Pseudococcus calceolaria (Maskell) (as *Dactylopius calceolaria*)	Hemiptera: Pseudococcidae	Maskell 1879	Citrophilus Mealy Bug:, feed by sucking sap from the host
Pseudocoremia colpogramma (Meyr.) (as *Selidosema colpogramma*)	Lepidoptera: Geometridae	Meyrick 1936	Looper
Tephritis sp.	Diptera: Tephritidae	Tillyard 1926	Fly
Tephrititis cassiniae Mall.	Diptera: Tephritidae	Malloch 1931	Fly

Table 2 Parasites recorded on '*Cassinia leptophylla*'.

species	order and family	literature	remarks (common name, distribution, etc.)
Athysanus negatus F.B. White	Homoptera	Myers 1922	
Cyoerobia carectorum Bergr.	Heteroptera	Bergroth 1927	
Graphania homoscia (Meyr.) (as *Melanchra homoscia*	Lepidoptera: Noctuidae	Hudson 1928	noctoid moth
Harmologa sisyrana Meyr.	Lepidoptera	Hudson 1928	
Heliostibes atychioides (Butl.)	Lepidoptera: Glyphipterygidae	Hudson 1928	Gregarious Tineid:
Liothula omnivora Fered. (as *Oeceticus omnivora*)	Lepidoptera: Psychidae	Hudson 1928	Bag Moth, host plants are exotic trees and manuka, kanuka, *Ozothamnus* and *Dracophyllum*
Novothymbris cassinae (Myers) (as *Diedrocephala cassiniae*)	Hemiptera: Cicadellidae	Myers 1923	Leafhopper, common in vast numbers on '*C. leptophylla*' and *Olearia solandri* on the sea coast, Wellington
Nysius sp.	Hemiptera: Lygaeidae	Myers 1926	common in the Wellington district, confined to '*C. leptophylla*'
Nysius huttoni F.B. White	Hemiptera: Lygaeidae	Myers 1926	Wheat Bug, Canterbury and Otago common, widely distributed throughout both Islands
Oemona hirta (F.)	Coleoptera: Cerambycidae	Hudson 1934	Lemon Tree Borer
Pseudocoremia rudisata (Walk.) (as *Selidosema rudisata*)	Lepidoptera: Geometridae	Hudson 1928	Looper

Table 2 continued

species	order and family	literature	remarks (common name, distribution, etc.)
Rhypodes clavicornis (F.) (as *Nysius clavicornis*)	Hemiptera: Lygaeidae	Myers 1926	
Saissetia cassiniae (Mask.) (as *Lecanium cassiniae*)	Hemiptera: Coccidae	Maskell 1891	Scale Insect, on '*C. leptophylla*', Wellington, Wairarapa, Hawke's Bay, and probably elsewhere
Saissetia oleae (Ol.) (as *Lecanium oleae*)	Hemiptera: Coccidae	Maskell 1985	Black Scale
Tephritis cassiniae Mall.	Diptera: Tephritidae	Harrison 1959	Fly
Trioza acuta (Ferris & Klyver)	Hemiptera: Psyllidae	Ferris & Klyver 1932	Lerp Insect, Psyllid: these small insects are like aphids in that they feed by sucking sap from their host plants but look like miniature cicadas (Scott 1984)
Zermizinga indocilisaria Walk. (as *Hybernia indocilis*)	Lepidoptera	Clark 1935	Moth
Zygina zealandica (Myers) (as *Erythroneura zealandica*)	Hemiptera: Cicadellidae	Myers 1923	Leafhopper

Table 3 Parasites recorded on '*Cassinia retorta*'.

species	order and family	literature	remarks (common name, distribution, etc.)
Oemona hirta (F.)	Coleoptera: Cerambycidae	Kirk 1896	Lemon Tree Borer

Table 4 Parasites recorded on '*Cassinia vauvilliersii*'.

species	order and family	literature	remarks (common name, distribution, etc.)
Eriophyes sp.	Acari: Eriophyidae	Moar 1958 in Lamb 1960	Witches' broom causing gallmite, Brtothers Ra, Canterbury
Harmologa columella Meyr.	Lepidoptera	Hudson 1939	
Pseudocoremia colpogramma (Meyr.) (as *Selidosema colpogramma*)	Lepidoptera: Geometridae	Hudson 1939	Looper
Pseudocoremia dejectaria (Walk.) (as *Selidosema dejectaria*)	Lepidoptera: Geometridae	Hudson 1939	Looper

Table 5 Parasites recorded on '*Cassinia fulvida*'.

species	order and family	literature	remarks (common name, distribution, etc.)
insect	?	Lush 1948 in Lamb 1960	Monothalamous bud galls, Christchurch
Selidosema adusta	Lepidoptera: Geometridae	Molloy 1959	Looper
Cecidomyid	Diptera: Cecidomyiidae	Molloy 1959, Sheppard 1965	Gall Midges

Table 5 continued

species	**order and family**	**literature**	**remarks (common name, distribution, etc.)**
species with affinities to *Dolichotetranchyus ancistrus*		Sheppard 1965	mite found in cavities of stem
Anthomyid Dipteran	Diptera: Anthomyiidae	Sheppard 1965	Seed Fly, collected by C. J. Burrows
undescribed Melolonthid beetle	Coleoptera: Scarabaeidae sub-family Melolonthinae	Sheppard 1965	beetle eating leaves of '*Cassinia*' and a grub eating shrub roots (including '*Cassinia*) found by P.M. Johns
Psyllid species similar to *Trioza acuta* (Ferris & Klyver)	Hemiptera: Psyllidae	Sheppard 1965	Lerp Insect

Appendix 3

Collecting data

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
1	11-02-98	P29 863206	Marlborough	Chalk Range,above Zoo swamp	750	shrub-/grassland	limestone		branchlets slender to stout	A-G	dominant	very polymorphic	J.M. Ward, JW 98009
2	11-02-98	P30 900159	Marlborough	above Remuera Station, Kekerengu Road	300	farm-/grassland	limestone	100-200		A-F	dominant	very polymorphic	J.M. Ward, JW 900159
3	12-02-98	P29 084286	Marlborough	Ward Beach, mouth of Flaxbourne River	sea level	sand dune	limestone/ sand	30-100		A-F	dominant	polymorphic, only small leafed	J.M. Ward, JW 98016
4	21-02-98	K34 092958	Canterbury	Chilton valley, Cass	600	shrubland		30-80		A-M	dominant	big leaves but yellow	I. Schönberger
5	07-03-98	N33 124062	N Canterbury	North of Motunau, near Greta Canyon (HW1)	180	roadside		80	branchlets slender, scraggly	A	1	very homogeneous (small leaved)	I. Schönberger
6	07-03-98	N33 232137	N Canterbury	5 km north of Hurunui bridge (HW1)	70	paddock/roadside		50-200	branchlets slender, scraggly	A-N	50-80	very homogeneous (small leaved)	I. Schönberger
7	08-03-98	N33 194077	N Canterbury	between Napenape and Motunau Beach, Blythe Rd.	160	roadside (unsealed)		80-100	branchlets slender, scraggly	A-E	10	pop. spread	I. Schönberger
8	08-03-98	N33 168078	N Canterbury	Stonyhurst Road (between HW1 and Stonyhurst)	100	roadside		80-100	branchlets slender, scraggly	A-G	>100	pop. spread, D-G further away	I. Schönberger
9	04-03-98	M27 617972	NW Nelson	Garibaldi Ridge, NE facing slope, unnamed peak (1430m)	1320	Tussock grassland along ridge, *Chionochloa pallens*, *Hebe* sp., *Dracophyllum* sp.		10-30	branchlets prostrate (ends decumbent)	A	frequent (on ridge)	plants all very small a. prostrate, pop. in fl., few fl. pre.	A. D. Wilton, ADW 98129
10	10-03-98	cultivated material in the glass house, no information about locality											2109
11	04-04-98	M31 606695	N Canterbury	W-end of St. James walkway, Lewis Pass, Tarn	863	shrub-/grassland		50-100	branchlets stout	A-D	50-80		I. Schönberger
12	04-04-98	N32 925365	N Canterbury	Lewis Pass (HW7) between Montrose and Lewis Pass	280	roadside/paddock		80-100	branchlets slender, scraggly	A-D	10		I. Schönberger
13	05-04-98	K33 913111	N Canterbury	Otira Valley, Southern Alps	1000	mixed herbfield/shrubland on damp n-facing slope beside track				A-C		homogeneous	R. McKenzie
14	08-04-98	E43 582293	N Southland	Mataura Valley	650	tussock grassland on damp slope				A-C		homogeneous	R. McKenzie
15	19-02-96	K34 074844	Canterbury	upper Cave Stream, between West Coast Rd. and Helicopter Hill	830	scrub, *Dracophyllum* sp.				A-B			R. McKenzie, RMcK
16	11-04-98	D40 162904	Fiordland	Hollyford Valley	700	shrubland in valley floor				A-D		homogeneous	R. McKenzie
17	15-04-98	C42 740575	Fiordland	Lake Wapiti	900	grass-/shrubland (*Chinochloea pallens*, *Hebe* sp.)	granit gravel			A	rare		D. Glenny 7484
18	18-04-98	P31 823887	Kaikoura Coast Marlborough	Waipapa Bay, beside railway (HW1)	5	coastal rocks		100-200	slender, scraggly	A-D	80	very homogeneous (small leaved)	I. Schönberger
19	18-04-98	P31 823891	Kaikoura Coast Marlborough	beside (HW1)	8	hillside covered with *Ozothamnus*, paddock		80-200	slender, scraggly	0	200-400 abundant	very homogeneous (small leaved)	I. Schönberger
20	18-04-98	P30 865955	Kaikoura Coast Marlborough	HW1 north of Clarence	5	roadside		100	slender, scraggly	0	1	small leaved	I. Schönberger
21	18-04-98	P30 943123	Kaikoura Coast Marlborough	HW1 3 km north of Kekerengu	5	roadside/paddock		80-200	slender, scraggly	0	100-300 abundant	very homogeneous (small leaved)	I. Schönberger

Collecting data continued

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
22	18-04-98	P29 007202	Kaikoura Coast Marlborough	HW1 1-3 km north of Wharanui	5	roadside/paddock		80-200	slender, scraggly	0	100-300 abundant	very homogeneous (small leaved)	I. Schönberger
23	18-04-98	P29 078298	Kaikoura Coast Marlborough	between Ward and Ward Beach	5	roadside/farmland/paddocks	limestone	80-100	slender, scraggly	0	abundant	only small leaved, but different in colour	I. Schönberger
25	18-04-98	P29 083288	Kaikoura Coast Marlborough	Ward Beach	2	Limestone Hill at beach	limestone rock	50-100	slender, scraggly to stout	A-E	abundant	only small leaved, but different in colour	I. Schönberger
26	18-04-98	P29 085286	Kaikoura Coast Marlborough	Ward Beach	sea level	sand dunes	sand/lime-stone	30-80	slender to stout	A-D	abundant	only small leaved, but different in colour	I. Schönberger
27	18-04-98	P30 888155	Marlborough	Zigzag above Remuera Station Kekerengu Road	450	farmland, paddock with *Kunzea ericoides* and *Leptospermum scoparium*		100-200	slender (at lower alt.) to stout (at higher alt.)	0	abundant	single specimen on roadside to dominant in higher altitude	I. Schönberger
28	18-04-98	P30 866176	Marlborough	Zigzag above Remuera Station Kekerengu Road	620	farmland, paddock with *Kunzea ericoides* and *Leptospermum scoparium*		80-200	polymorphic, slender to stout	A-D	abundant	extreme polymorphic	I. Schönberger
29	18-04-98	P30 866188	Marlborough	Zigzag above Remuera Station Kekerengu Road	616	farmland, paddock with *Kunzea ericoides* and *Leptospermum scoparium*		80-200	polymorphic, slender to stout	A	abundant	extreme polymorphic	I. Schönberger
30	18-04-98	P30 868199	Marlborough	Zigzag above Remuera Station Kekerengu Road	720	farmland, paddock with *Kunzea ericoides* and *Leptospermum scoparium*		80-100		A-C	abundant	extreme polymorphic	I. Schönberger
31	19-04-98	P30 791996	Marlborough	Georg stream/Clarence River	100	roadside, farmland, shrubland		80-100	scraggly, slender, unhealthy	A-B	(50) occasional	homogeneous, small leaved	I. Schönberger
32	19-04-98	O31 569739	Marlborough	Mt Fyffe	600	road-/track-side, grass-/shrubland		30-50	slender to stout, unhealthy	A-C	occasional	mixture with large leaved and small leaved specimens	I. Schönberger
33	19-04-98	O31 575745	Marlborough	Mt Fyffe	760	road-/track-side, grass-/shrubland		30-50	very stout	A-B	frequent	mainly large leaved specimens	I. Schönberger
34	23-04-98	J37 653952	S Canterbury	Orari-River Road towards Mt Peel	300	roadside		50-100	scraggly, slender, unhealthy	A	10 occasional	homogeneous	I. Schönberger
35	23-04-98	J37 685005	S Canterbury	Peel Forest Park, Deer Spur Track, 5 km NW of tarn, Mt Peel	705	bush, shrubland, track side		20	very small	A	rare at this altitude	first specimen at this altitude	I. Schönberger
36	23-04-98	J37 685005	S Canterbury	Peel Forest Park, Deer Spur Track, Mt Peel	710	bush, shrubland, track side		20	very small	A	rare at this altitude	3 specimens within a few hundred meters	I. Schönberger
37	23-04-98	J37 685008	S Canterbury	Peel Forest Park, Deer Spur Track	740	shrubland, track side		50	taller and healthier	A-B	rare at this altitude		I. Schönberger
38	23-04-98	J37 684009	S Canterbury	Peel Forest Park, Deer Spur Track	770	shrub-/grassland above tree line		50	taller and healthier, leaves bigger	A	common		I. Schönberger
39	23-04-98	J37 684010	S Canterbury	Peel Forest Park, Deer Spur Track	790	shrub-/grassland above tree line		50		A	common		I. Schönberger
40	23-04-98	J37 683011	S Canterbury	Peel Forest Park, Deer Spur Track	820	shrub-/grassland above tree line		50	small round shrubs, healthy	A-D	abundant		I. Schönberger
41	25-04-98	G39 410419	Central Otago	Ben Avon homestead: 1 specimen cultivated in garden	800	cultivated		80	stout	A	rare to absent		I. Schönberger

Collecting data continued

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
42	25-04-98	G41 215805	Central Otago	Bendigo	386			30-50	very scraggly, slender	A-B	rare	leaf margin recurved, small leaved	I. Schönberger
43	25-04-98	G41 176792	Central Otago	Clutha Valley near Crippletown	240	between rocks on cliffs beside road		30-50	very scraggly, slender	A-C	rare	leaf margin recurved, small leaved	I. Schönberger
44	26-04-98	F41 875715	Otago	Mt Scott, Crown Range	800	grassland with *Aciphylla* sp.		50	branchlets stout, roundish, ∅ 50-100 cm	A-C	rare	big leaved	I. Schönberger
45	26-04-98	G41 106675	Otago	Cromwell, village end	250	cultivated		50-80	branchlets slender to stout	A	rare	biggish leaves	I. Schönberger
46	26-04-98	G42 202522	Otago	between Cromwell and Clyde, Gold Monument	240	cultivated Car park!				A-D	10	very polymorphic	I. Schönberger
47	26-04-98	G42 205355	Otago	9,1 km south of Butchers Dam (to Fruitland)	380	paddock		100-200	very scraggly, slender	A-D	10	leaf margin recurved, small leaved	I. Schönberger
48	26-04-98	N32 999572	Canterbury	Hanmer Range, Jollies Pass	700				polymorphic	A-F	occasional	polymorphic	A. D. Mitchell
49	30-04-98	L35 136472	S Canterbury	Hood Bush, west of Whitecliff, Whitecliff Road	540-560	reserve, shrub, grassland	limestone	20-50	only small shrubs	A-B	occasional	homogeneous	I. Schönberger
50	30-04-98	K35 917606	S Canterbury	Algidus Road to Lake Coleridge/Rakaia river	580	roadside with *Agrostis capillaris*		50-80	shrub roundish, healthy	A	occasional		I. Schönberger
51	30-04-98	K36 932387	S Canterbury	Mt Hutt Skifield Road, 1,4 km from Ski-ticket hut	640	bank, shrubland,		40	not flowering, unhealthy	A	rare to absent		I. Schönberger
52	3-05-98	M36 898380	Canterbury	South Brighton, The Spit	2	on sand dune	sand	50-80	scraggly, unhealthy	A-D	occasional	small leaved	I. Schönberger
53	10-05-98	M34 563823	Canterbury	Mt Thomas, top of Wooded Gully track	940	shrubland with *Coprosma microcarpa*, *Chionochloa rubra*	greywacke, loess			A-B	frequent		D. Glenny 7496
54	13-05-98	P28 972568	Marlborough	Weld Pass, HW1, paddock on roadside	200	paddock, between shrubs		50-200	scraggly, more or less slender	A-J	abundant	very polymorphic	I. Schönberger
55	13-05-98	P28 812554	Marlborough	Brancott, Station of Ben Wadworth, nw of Blenheim	>400	farmland, shrub- and grass land		80-200	slender (lower alt.) to stout (higher alt.)	A-AJ	dominant	very polymorphic	I. Schönberger
56	13-05-98	P30 865965	Kaikoura Coast Marlborough	HW1 between Kekerengu and Clarence, 5 km north of bridge	30	roadside		30-200	slender, scraggly	A-D	occasional	homogeneous	I. Schönberger
57	13-05-98	N33 141075	Canterbury	between Hurunui River Bridge and Davar	100	paddock		50-200	slender, scraggly	A-E	80-100	very homogeneous	I. Schönberger
58	13-05-98	Y18 565710	Gisborne	Makorori beach, 10 Min north of Gisborne	sea level	mudstone, 2 m above high tide mark on south-facing cliff	mudstone			A-B		homogeneous	T. Jenkins
59	16-05-98	I37 090858	S Canterbury	Lake Tekapo, Church of good shepherd	750	cultivated in garden		50-80	round bushes, slender	A	3	homogeneous	I. Schönberger
60	16-05-98	I38 980725	S Canterbury	HW8 between Lake Tekapo a. Lake Pukaki	520	*Hieracium pilosella* desert, paddock		50-60	compact, stout	A-B	2	homogeneous	I. Schönberger
61	16-05-98	H38 869672	S Canterbury	first, former parking place at Lake Pukaki	560	maybe cultivated		50-60	compact, stout		5	homogeneous	I. Schönberger
62	16-05-98	H38 805605	S Canterbury	HW80, west side of Lake Pukaki and dam	500	roadside, shrub and grassland		50-100	compact, round	A-C	frequent	homogeneous	I. Schönberger

Collecting data continued

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
63	16-05-98	H38 806761	S Canterbury	Peter' s Lookout (Lake Pukaki)	580	grass- and shrubland		80-100	compact and round, stout	A-B	frequent	homogeneous	I. Schönberger
64	17-05-98	H36 763136	S Canterbury	Mt Cook National Park: Red Tarn	1080	grassland		30-80	compact	A-E	frequent	homogeneous	I. Schönberger
65	31-05-98	N29 943313	Nelson	Mt Robert Ski Field Rd (Lake Rotoiti)	880	shrubland between *Leptospermum scoparium*		10-30	small	A-C	occasional	homogeneous	I. Schönberger
66	31-05-98	N29 022220	Nelson	Wairau River Valley, Rainbow Ski Area	780	roadside, shrubland		50-80	small	A-D	occasional	homogeneous	I. Schönberger
67	31-05-98		Marlborough	Wairau River, main road Nelson – Blenheim (HW63)		*Leptospermum scoparium* shrub		50-80	healthy	A-F	frequent	polymorphic	I. Schönberger
68	31-05-98	O29 427366	Marlborough	Waihopai River, end of gravel road	520	farmland, paddock		30-80		A-E	common	homogeneous	I. Schönberger
69	30-06-98	L34 491784	Canterbury	Mt Richardson	400					A			B. Braun
70	18-01-98	R27 624094	Wellington	Te Korohiwa Rocks, Titahi Bay	2	on greywacke, cliff faces, *Crasspedia uniflora var. maritima*	greywacke			A	common		I. Breitwieser 2072
71	17-01-98	Z14 992753	Gisborne	East Cape at East Cape Lighthouse	144					A	common		I. Breitwieser 2069
72	13-01-98	Y19 338343	Gisborne	North of Mahiha (North of Mahanga)	60	coastal cliff				A			I. Breitwieser 2067
73	03-95	H36 805197	S Canterbury	Mt Cook National Park: Tasman Glacier, Blue Pools	800	mixed herbfield, shrubland, *Aciphylla* sp.				A	common		A. Bresinsky
74	22-02-98	K33 913111	Westland	Otira Valley, Southern Alps	1000	mixed herbfield, shrubland		20-80	compact	A-B	common		I. Schönberger
75	09-09-98	K33 913111	Westland	Otira Valley, Southern Alps	1000	mixed herbfield, shrubland		20-80	compact	0	common		I. Schönberger
76	02-09-98	M26 557342	Nelson	Heaphy Track, Gouland Downs (betw. Perry Saddle & Goulan-H.)	650	tussock (*Chinochloea rubra*)		10-30	compact, leaves dense, prostrate	A-L	common		I. Schönberger
77	27-06-98 28-05-00	M35 869505	Canterbury	Between Spencer Park and Waimairi Beach	sea level	sand dunes	sand	50-80	scraggly, wide	A-C	occasional		I. Schönberger
78	29-12-95	N30 233934	Marlborough	Barefell Pass, tussock grassland near 4WD track	1280	tussock grassland				A-H	very common		R. McKenzie, RMcK 162/1-8
79	03-09-97	O28 44_70_	SE Nelson	Johnson Peak, Richmond Range, between J. Peak and Mt Fell	1550	tussock grassland on NE facing slope near ridge top				A	not common		R. McKenzie, RMcK 325
80	17-01-98	R27 541818	Wellington	Red Rock, Cook Strait	15	coastal bluff system above stream that enters beach	coastal rock			A	frequent on rocks		A. D. Wilton, ADW 98032
81	20-01-98	R28 700728	Wellington	Turakirae Head, Cook Strait	sea level	open coastal scrub-grassland, *Luzula*, *Craspedia*, *Gnaphalium*	coastal rock			A	common		A. D. Wilton, ADW 98036
82	31-01-98	E43 516241	N Southland	Mt Eyre	650	sparsely vegetated, stony, NE facing slope				A-C			R. McKenzie, RMcK 388/1-3
83	31-01-98	E43 49_23_	N Southland	Mt Eyre	1250	tussock grassland on ridge				A-B			R. McKenzie, RMcK 389/1-2
84	28-12-95	O30 32_98_	N Canterbury	Yeo Stream, Inland Kaikoura Range	930	rocky riverbank				A-B			R. McKenzie, RMcK 148/2+4
85	15-03-96	L34 112957	Canterbury	Sugar Loaf, Cass	1180	tussock grassland on SW-facing slope				A-C		A + B: dominant form, C: unusual	R. McKenzie, RMcK 269/1-3
86	03-05-96	N31 238602	N Canterbury	Cunningham Stream, Mt Terako	1050	beside stream among grass at base of SE-facing scree slope				A			R. McKenzie, RMcK 263
87	03-04-96	K33 926098	Canterbury	Temple Basin, Arthurs Pass, beside track to ski-field huts	950	tussock grassland/herbfield				A		A + B: dominant form, C: unusual	R. McKenzie, RMcK 255

Collecting data continued

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
88	03-05-96	N31 21_60_	Canterbury	Mt Lyford, beside road beside first (red) gate on Ski-field Road	1300	roadside				A			R. McKenzie, RMcK 256
89	10-04-98	E42 403338	N Southland	Gorgeburn Valley, Eyre Mts	950	tussock grassland/herbfield				A-B		Ro1	R. McKenzie
90	30-08-98	N32 968565	Canterbury	Hanmer Range, Waterfall Track	600	*Nothofagus* forest		20-30	small and unhealthy	A-G	occasional	different coloured leaves	I. Schönberger
91	18-10-98	K31 768786	Westland	Croesus track, Croesus Knob	1100	shrubland with *Chinochloea rubra*, *Dracophyllum* sp.		20	compact, leaves dense, prostrate	A	1 (rare)	see 9 and 76	I. Schönberger
92	18-10-98	L34 360 735	Canterbury	Mt Oxford, East Brand, Coopers Creek	540	*Poa sita*, *Agrostis capillaris*, river bed, tussock grassland	river gravel	<100		A	occasional		D. Glenny 7502
94	26-04-94		Wellington	Lyall Bay									
95	--96		Canterbury	Poverty Flat, Mt White Road									M. Dawson G302/96
96	12-8-93			Happy valley								plant died in the garden	
97	26-01-95	E40 362 949	Fiordland	Scotts Basin, Humboldt Mts	1159	mixed shrub-tussock							A. D. Wilton, ADW 310; CANU37748
98	23-04-97		Southland	Catlins State Forest Park									244/97
99	--96		N Canterbury	near St. James Walkway, Lewis Pass									M. Dawson 329/96
100	31-10-98	N36 027 203	Canterbury	Pigeon Bay Road above Kukupa	220	disturbed bank above road, *Kunzea ericoides*, *Pinus*	loess	80	unhealthy	A	1 (rare)	see CHR 494035	I. Schönberger
101	29-10-98	M24 857 773	Nelson	Cape Farewell, Approach from 4WD track to lighthouse, follow ridges to crest of rock outcrops east of the track	100								Garnock-Jones 2343, WELTU 19505
102	04-11-98	K36 763 324	S Canterbury	Mt Somers, near Woolshed Creek Walkway track	1025	mixed shrub, tussock grassland, NE-facing slope		30-120	upright	A-B	occasional		I. Schönberger
103	04-11-98	K36 770 319	S Canterbury	Mt Somers	1375	mixed shrub, tussock grassland, NE-facing slope		30-40	decumbent	A-B	rare (only 3)	A = white form, B= usual form	I. Schönberger
104	12-11-98	M02 814 524	N Auckland	Cape Reinga, N Cape, Coastal walk way, cliff edge	480	shrubland, *Leptospermum scoparium*, *Kunzea ericoides*		30	wind-shaped, scraggly	A-I	common	homogeneous	I. Schönberger
105	12-11-98	M02 814 497	N Auckland	Te Werahi Beach, N Cape	sea level	sand dunes	sand	80-100	round (∅ 1.50m), stout, dense, prostrate	A-O	common	homogeneous	I. Schönberger
106	12-11-98	N02 979 539	N Auckland	Hooper Point, N Cape	232	shrub, paddock, *Leptospermum scoparium*, *Kunzea ericoides*		40	∅ 30 cm, dense, stout, wind shaped	A-E	occasional	homogeneous	I. Schönberger
107	12-11-98	N02 974 520	N Auckland	Spirits Bay, N Cape	sea level	sand dunes	sand	80	∅ 100 cm, round, bit prostrate, stout	A-I	common	homogeneous	I. Schönberger
108	12-11-98	N03 188 005	N Auckland	Ninety Mile Beach, Hukatere, Fire Lookout	sea level	sand dunes	sand	80	∅ 100 cm, round, bit prostrate, stout	A-D	common	homogeneous	I. Schönberger
109	08-11-98	O31 424 755	Marlborough	Seaward Kaikoura Range near Cattle Spur (Hut)	920					A			B. Braun
110	14-11-98	M28 701 602	Nelson	Mt Owen, Bulmer Lake	1240	margin of *Nothofagus menziesii* forest, and *Chinochloea pallens*, *C. flavescens*, *Dracophyllum uniflorum*, tussock-grassland	marble-derived	19	erect, neat	A	occasional	homogeneous (only 3 seen)	D. Glenny 7504

Collecting data continued

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
111	20-11-98	I44 173 859	Otago	Dunedin, Sullivans Dam	300	*Leptospermum scoparium*, *Leycesteria formosa*, *Ulex europaeus*, *Cytisus scoparius*	Sandstone YBE	150	erect	A	occasional	homogeneous	D. Glenny 7512
112	19-11-98	P20 048 103	Taranaki	Mt Stratford ski field (Mt Egmont)	1100					A			A. Weiss & G. Eichinger
113	27-11-98	G39 123 438	C-Otago	Lake Hawea, Sawyer Burn, poled route	800					A			A. Weiss & G. Eichinger
114	29-11-98	H46 625 245	S Otago	3 km to Kaka Point (Nugget Point)	10	road side, farm land	loamy	100	erect, round bushes	A-B	occasional	homogeneous	I. Schönberger
115	29-11-98	I44 177 867	Otago	8.7 km to Dunedin, Kilmog Hill, HW1		road side, bunk	loamy	100	erect, round bushes	A-C	occasional	homogeneous	I. Schönberger
116	30-11-98	H46 545 157	S Otago	roadside betw. Balclutha a. Owaka	20	roadside	loamy	100	erect, round bushes	A-C	common	homogeneous	I. Schönberger
117	1-12-98	G47 319 942	Southland	Cathedral Cave car park	100	margin of bush	loamy	150	erect, round bushes	A-J	very common	homogeneous, red buds, white buds	I. Schönberger
118	1-12-98	G47 321 938	Southland	Cathedral Cave walk way	20	slope, bush margin	loamy	100	erect	A	occasional	homogeneous	I. Schönberger
119	26-11-98	L29 176 442	Nelson	Stockton Plateau, eastern edge	700	*Chionochloa rubra*, mixed tussock land	greywacke	40	prostrate	A	common	homogeneous	D. Glenny 7543
120	21-12-98	I40 989 001	Otago	St Marys Range	1050	mixed tussock, herbfield, shrubland	loam	100	upright, shrubby, dense	A-C	common	homogeneous	I. Schönberger
121	2-1-99	P28 978 795	Marlborough	Road between Rarangi and Robin Hood Bay	100	rocky roadside bank, among gorse a. manuka	rocky/clay	80	upright, bushy, dense	A	occasional	homogeneous	I. Schönberger
122	2-1-99	P27 995 825	Marlborough	Robin Hood Bay	20	roadside bank, among exotic grasses, seedlings common	loamy	100	upright, bushy, dense	A-C	local	homogeneous	I. Schönberger
123	2-1-99	P27 018 853	Marlborough	Ocean Bay, Port Underwood	20	roadside among exotic grasses	loamy	100-150	upright, shrubby, dense	A-B	local	homogeneous	I. Schönberger
124	5-1-99	H43 74_ 21_	Otago	Rock + Pillar Range near McPhee's Rock	1200	south facing slope in tussock grassland with Rhytidosperma sp. , Leucopogon fraseri		60	somewhat divaricating	A-D		some plants with reddish bud others whitish, otherwise homogeneous	Garnock-Jones 2359
125	9-12-98	F43 973 123	Southland	Above Titan Rocks track, Waikaia		tussock, *Chinochloea rigida*				A			B. Braun
126	30-11-98	N30 272 020	Marlborough	Mt Chisholm, Molesworth	1350	tussock grassland, herbfield	loam	30	low growing, shrubby	A	uncommon	homogeneous	R. McKenzie
127	21-1-99	N32 956 589	Marlborough	Jack's Pass		*Kunzea ericoides*, grassland	loam	50	round, compact bushes	A-C	common	homogeneous	I. Schönberger
128	22-1-99	O30 337 965	Marlborough	Yeo Stream		*Hieracium pilosella*, *Discaria tomatou*, *Aciphylla*, *Hebe*, shrubland		100	scraggly, unhealthy, dry	A-D	occasional	homogeneous	I. Schönberger
129	22-1-99		Marlborough	Yeo		s. 128		100	scraggly, unhealthy, dry	A-D	common	homogeneous	I. Schönberger
130	23-1-99	N32 999 569	Marlborough	Jollies Pass	820	tussock grassland, *Ozothamnus* shrubland	loam	30-80	dense	A-D	abundant	homogeneous	I. Schönberger
131	23-1-99	N32 001 562	Marlborough	Jollies Pass	700	*Pinus*, *Kunzea ericoides*, *Nothofagus*, grass, shrubs	loam	100	not very dense (more shady)	A-B	common	polymorphic, A large spreading corymbs, B dense corymbs	I. Schönberger
132	19-1-99	H43 750 218	Otago	Rock + Pillar Range	1285			low	low springy shrub, wind-shorn	A	common	homogeneous, slide!!	D. Glenny 7700
133	18-1-99	C44 732 806	Southland	Mt Burns	1100	*Chionochloa pallens*, *Phormium cookianum*, *Hebe*, just above treeline				A	abundant		D. Glenny 7696

Collecting data continued

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
134	19-1-99	I44 025 805	Otago	Danseys Pass	900	*Chionochloa rigida*, *Schoenus paucilorus* sedge tussock land on hillslope				A	common		D. Glenny 7704
135	16-1-99	E43 365 010	Southland	West Dome	640	*Leptospermum scoparium*, *Hebe salicifolia*, *Holcus lanatus*, *Anthoxanthum odoratum*, shrubland	sandstone mixture	100	very bushy	A			D. Glenny 7675
136	14-1-99	F39 075 358	Otago	Lake Wanaka and Lake Hawea, the Neck	1030	*Chionochloa rigida*, *Agrostis capillaris* tussock grassland on hillslope		50	bushy				D. Glenny 7658
137	14-2-99	P27 853 908	Marlborough	Anakiwa Road, Okiwa Bay, Queen Charlotte Sound	10	roadside, *Ulex europaeus*, *Rubus*, exotic grass	loam	100	scraggly	A	1	homogeneous	I. Schönberger
138	14-2-99	P27 863 928	Marlborough	Anawika, Thompson Bay, Queen Charlotte Sound	sea level	above the high tide zone, with exotic grasses	sand	150	a bit scraggly, healthy	A	1	homogeneous	I. Schönberger
139	14-2-99	P27 902 925	Marlborough	Queen Charlotte Drive, Grove Arm	40	coastal shrub/bush	loam	80	very scraggly, unhealthy	A-C	occasional	homogeneous	I. Schönberger
140	14-2-99	P27 875 922	Marlborough	Queen Charlotte Drive	20	coastal shrub/bush	loam	100	scraggly, untidy	A-B	occasional	homogeneous	I. Schönberger
141	14-2-99	P27 892 810	Marlborough	Parara Wetland, HW1	45	roadside bank	loamy, rocky	150	healthy	A-B	occasional	homogeneous	I. Schönberger
142	14-2-99	P28 964 587	Marlborough	HW1, south of Blenheim, Pukapuka Stream	80	cow paddock, next to stream	loam	100	scraggly, unhealthy, parasites	A-D	common	polymorphic	I. Schönberger
143	14-2-99	P28 970 569	Marlborough	Weld Pass	200	grassland, paddock	rocky loam	80	scraggly, unhealthy, parasites	A-F	abundant	polymorphic	I. Schönberger
144	02-06-99	C44 732 817	Fiordland	Borland Saddle	1050	well drained, *Chionochloa*, *Celmisia*		100	∅ 150 cm	A	common	homogeneous	A. McCall
145	28-1-99	H36 747 174	S Canterbury	Mt Cook National Park	1100	tussock, *Chionochloa flavescens*, *Ranunculus lyallii*, *Celmisia*		80	scraggly	A	common	homogeneous	A. McCall
146	08-02-99	S20 277 085	Wellington	Ruapheu,Turoa Skifield	1560	In mixed shrub tussock community on ash deposits being eroded by numerous watercoarses. *Chionochloa rubra*; *Coprosma* sp; *Dracophyllum recurvum*; *Bracyglottis bidwillii*; *Celmisia spectabilis*		40-50		A			A. D. Wilton, ADW 99113
147	09-02-99	T20 463 165	Wellington	Ruapheu,Rangipo Desert	960	In shurb/tussock community c. 1 km along Rangipo Intake Rd, *Dracophyllum uniflora*; *Chionochloa rubra*; *Kunzea ericiodes*; *Hebe* sp; *Muhlenbeckia axillaris*; *Raoulia. australis*; *R. albosericea*; *Leucopogon fraseri*; *L. colensoi*; *L. emptrifolia*		20-50		A			A. D. Wilton, ADW 99120
148	10-02-99	P20 003 185	Taranaki	Pouaki Range	1160	near track to tarns to N of Pouaki Hut, in Olearia/tussock community, *Luzula banksiana* var. *migrata*; *Gahnia* sp.; *Olearia* sp.; *Dracophyllum filifolium*; *Bulbinella* sp; *Chionchloa rubra*; *Coprosma* sp.; *Hebe* sp; *Anaphalioides alpina*				A			A. D. Wilton, ADW 99133
149	08-03-99	O29 69_25_	Marlborough	Isis Stream (Awatere River)	520	*Discaria tomatou*, paddock	loamy	20-200	bushy, compact, dense - scraggly		abundant	very polymorphic	I. Schönberger
150	23-02-99	M28 735 649	Nelson	Mt Owen, Granity Pass	1180								D. Glenny

Collecting data continued

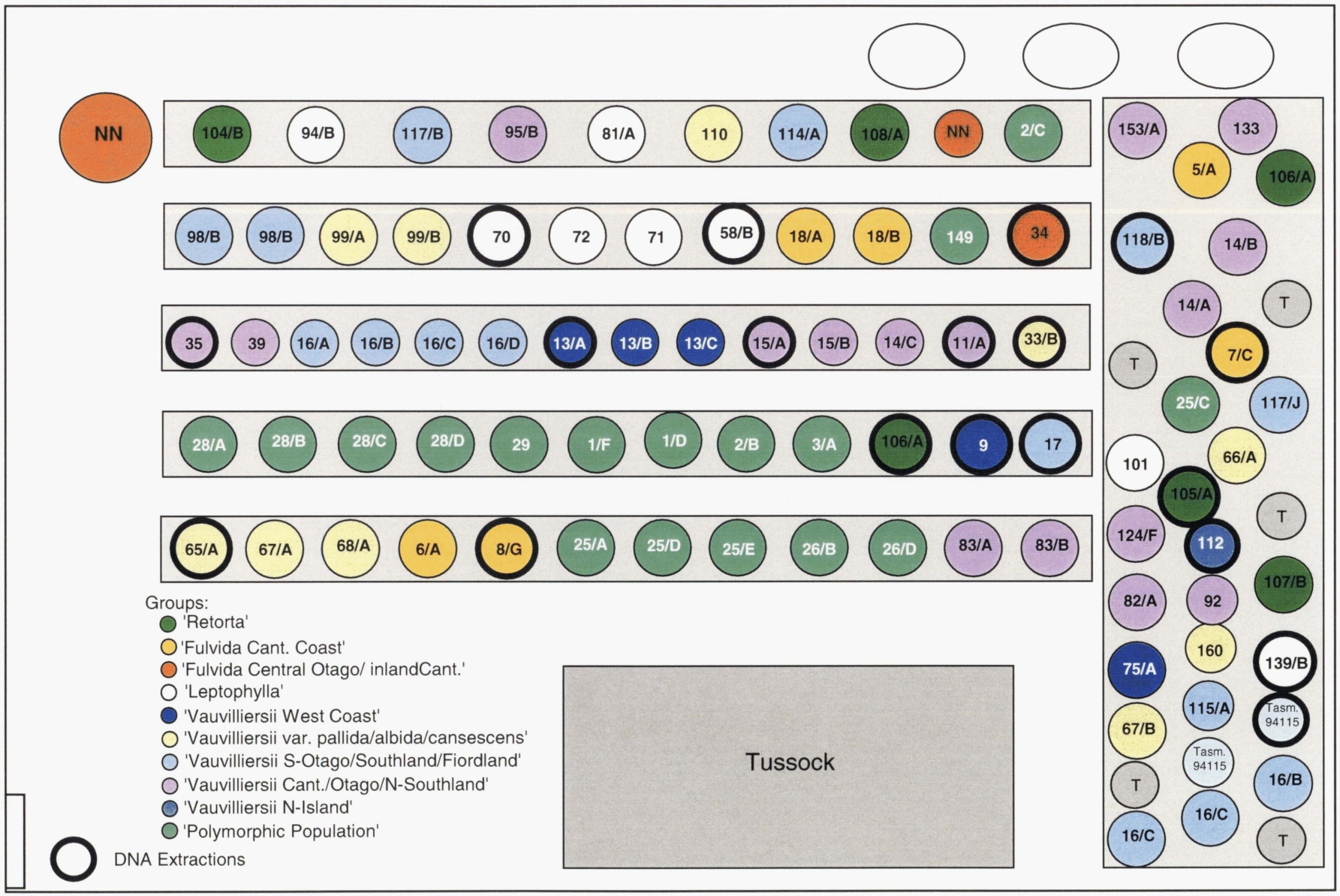
No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
151	19-03-99	K34 973 945	Canterbury	Mt Bruce	1340	*Hebe* sp., *Chionochloa rubra*, *Pinus radiata*	wet	120		A	occasional	polymorphic	A. McCall
152	25-03-99	C42 882 342	Fiordland	Takahe Valley, Munkisa Mts.	1160	*Dracophyllum* sp., *Hebe* sp.		120		A-C	occasional	homogeneous	A. McCall
153	11-12-98	M27 743 093	NW Nelson	near Mt Mytton	1570	alpine, exposed, tussock/herbfield, ridge, south facing, *Hebe coarctata*, *Chionochloa pallens*, *Celmisia bellidifolia*		18	spreading outwards, semi prostrate	A-B		polymorphic	K. A. Ford 568-9/98
154	13-12-98	M24 873 776	Nelson	Puponga Farm, Farewell Spit (base)	5	coastal limestone bluffs above beach, growing on big boulders, west facing, *Phormium tenax*, *Coprosma robusta*	limestone bluffs			A			K. A. Ford 570/98
155	31-12-98	I41 027 806	Otago	Dansey's Pass, new top at Pass	900	tussock grassland, *Chionochloa rigida*, *Bulbinella* sp.		50					K. A. Ford 175/98
156	17-04-99	J37 675 015	S Canterbury	Little Mt Peel	1090	shrubland		80	scraggly	A-C	occasional	homogeneous	G.J. Houliston
157	26-04-99	P25 836 387	Marlborough	D'Urville Island, Attempt Hill	520	edge of Rimu forest, *Nothofagus menziesii*, *Kunzea ericoides*		50	healthy	A-C	occasional	homogeneous	M. Todd
158	28-04-99	N02 11_ 55_	N Auckland	Surville Cliffs		cultivated				A		cultivated	Smith, Percy's Reserve
159	28-04-99	P29 933 205	Marlborough	Ben More		cultivated				A		cultivated, Tony Druce	Smith, Percy's Reserve
160	18-01-99	L31 455 603	Westland	Blue-Grey River near Mt Boscowen	1140	subalpine shrubland, slope, *Chionochloa pallens*, *Aristotelia fruticosa*, *Phormium cookianium*, *Polystichum vestitum*, *Blechnum montanum*, *Hoheria glabrata*				A			K. A. Ford
161	24-09-99	N31 298 780	N Canterbury	Clarence River flats, upstream of Palmer Stream	570	river terrace of *Ozothamnus*, *Cytisus scoparius*, *Anthoxanthum odoratum* shrubland		100		A		homogeneous	Glenny 7871
162	15-01-00	DEF48 137528	Stewart Island	Mason Bay between Freshwater Hut and Mason Bay	60					A	occasional	homogeneous	I. Breitwieser & R. Vogt 2120
163	12-01-00	M32 585 466	Canterbury	Confluence of Hope and Boyle Rivers, Lake Sumner Forest Park	540	Scrubby, *Discaria tomatou*, *Leptospermum scoparium*, *Kunzea ericoides*	greywacke	>100	tidy, compact	A-C	occasional	homogeneous	A. McCall
164	12-03-00	N36 920 359	Canterbury	Taylors Mistake, Christchurch	1	Sand dune	sand	40-80	unhealthy, scraggly, dry	A	7	homogeneous	I. Schönberger
165	11-03-00	U20 073 088	Hawkes Bay	Kaweka Range near lookout, off Kaweka Road	1060	subalpine scrub				A	common	homogeneous	M. Bayly, MJB 1338
166	11-03-00	W19 670 286	Hawkes Bay	Mohaka River near crossing on HW 2	60	Mudstone embankment beside road	mudstone		very tall	A	common	homogeneous	M. Bayly, MJB 1345
167	12-03-00	Z17 721 924	Gisborne	Waihau Bay, beside Waihau Road	60					A	common	homogeneous	M. Bayly, MJB 1358
168	13-03-00	X15 13_ 62_	Gisborne	Beside Highway 35 near Tokata Point						A	common	homogeneous	M. Bayly, MJB 1368
169	14-03-00	T20 46_07_	Wellington	Volcanic Plateau, Rangipo Desert beside Desert Road	1060					A	common	homogeneous	M. Bayly, MJB 1375
170	16-03-00	E47 528 591	Southland	Bluff Hill near lookout at summit	265	Shrubland, *Olearia arborescens*, *Pittosporum tenuifolium*, *Pseudopanax* sp.				A	common	homogeneous	R. McKenzie

Collecting data continued

No.	Date	NZMS 260	Region	Locality	Alt. (m)	Habitat and assoc. spp.	Soil/ Substrate	average Plant size	Habit (aspect, shape)	Samples	Abundance	Remarks on population	Collector
171	30-03-00	P19 845 287	Taranaki	Taranaki, near the end of Leith Road	15	Beside road on walls of bank				A	common	homogeneous	M. Bayly, MJB 1398
172	31-03-00	S26 974 383	Wellington	Otaki River Valley	100	Paddock beside road				A	common	homogeneous	M. Bayly, MJB 1399
173	14-04-00	O30 627 149	Marlborough	Hodder River near Hodder Hut, Inland Kaikoura Range	1450	Mixed grassland		60	bushy, compact	A	common	homogeneous	R. McKenzie
174	15-04-00	O30 630 191	Marlborough	Hodder River, Inland Kaikoura	775	Scrub beside riverbed		125	straggly, bushy	A, B	common	polymorphic	R. McKenzie
175	18-04-00	D48 165 776	Stewart Island	Smoky beach	20	sand dune	sand	20	short, compact	A	occasional	homogeneous	A. McCall
176	18-04-00	D48 083 642	Stewart Island	Hellfire Pass	220	Coastal cliff/sand	sand	220		A	occasional	homogeneous	A. McCall
177	14-05-00	L34 804 805	Canterbury	Mt Richardson	1040	Subalpine shrub, *Dracophyllum* sp., *Chinochloea* sp.		100	scraggly-compact	A-C	occasional	homogeneous	A. McCall
Cass	27-06-99	K34 089 962	Canterbury	behind Cass Field Station	600	*Ozothamnus* shrub-grassland	strongly leached yellow-brown earth overlying gravel	70-90	upright, round, more or less compact with stout shoots	1-12	dominant	homogeneous	I. Schönberger
CHR 389520	01-01-82	N02 11- 55-	N Auckland	Northwest of North Cape, Surville Cliffs	180								A.P. Druce
CHR 355755	18-01-79	N02 12- 55-	N Auckland	North Cape near serpentine quarry		shrubland					common		D. R. Given 11558; J. Bartlett
Cassinia aculeata 94115	20-02-00		Tasmania	Mt Hartz National Park									J.M. Ward, JW 94115
O. rodwayi 94155	14-04-00		Tasmania	Mt Field National Park									J.M. Ward, JW 94155

Appendix 4

Experimental Garden (Garden Plan)



Experimental Garden

Table 1 Living plant material kept in the experimental garden and glasshouse.

No.	Date	NZMS 260	Region	Locality	Herbar	Garden	Glasshouse	Collector	Col. No.
1	11/02/98	P29 863206	Marlborough	Chalk Range,above Zoo swamp	A-G	D, F	-	JW	98009
2	11/02/98	P30 900159	Marlborough	above Remuera St., Kekerengu Road	A-F	B, C	B	JW	900159
3	12/02/98	P29 084286	Marlborough	Ward Beach, mouth of Flaxbourne River	A-F	A	A	JW	98016
5	07/03/98	N33 124062	N Canterbury	N of Motunau, near Greta Canyon (HW1)	A	A	-	ISch	
6	07/03/98	N33 232137	N Canterbury	c. 5 km n of Hurunui bridge (HW1)	A-N	A	A	ISch	
7	08/03/98	N33 194077	N Canterbury	betw. Napenape a. Motunau Beach	A-E	C	C	ISch	
8	08/03/98	N33 168078	N Canterbury	Stonyhurst Road (betw. HW1 a. Stonyhurst)	A-G	A G	A, G	ISch	
9	04/03/98	M27 617972	NW Nelson	Garibaldi Ridge, NE facing slope	A	A	A	ADW	98129
11	04/04/98	M31 606695	N Canterbury	W-end of St. James WW, Lewis Pass	A, B, D	A	A	ISch	
13	05/04/98	K33 913111	Westland	Otira Valley, Southern Alps	A, B, C	A B C	A, B, C	RMcK	
14	08/04/98	E43 582293	N Southland	Mataura Valley	A, B, C	A, B, C	A, C	RMcK	
15	10/04/98	E42 403338	N Southland	Gorgeburn Valley, Eyre Mts	A, B	A B	A, B	RMcK	
16	11/04/98	D40 162904	Fiordland	Hollyford Valley	A, B, C, D	A, B, C, D	A, B, D	RMcK	
17	15/04/98	C42 740575	Fiordland	Lake Wapiti	A	A	A	D. Glenny	7484
18	18/04/98	P31 823887	Kaikoura Coast/Marlborough	Waipapa Bay, beside railway (HW1)	A, B, C, D	A, B	A	ISch	
25	18/04/98	P29 083288	Kaikoura Coast/Marlborough	Ward Beach	A-E	A, C, D, E	A, C, D	ISch	

DNA Extraction

Table 1 continued

26	18/04/98	P29 085286	Kaikoura Coast/Marlborough	Ward Beach	A, B, C, D	B, D	A, B, D	ISch	
28	18/04/98	P30 866176	Marlborough	Zigzag above Remuera Station Kekerengu Road	A, B, C, D	A, B, C, D	B, D	ISch	
29	18/04/98	P30 866188	Marlborough	Zigzag above Remuera Station Kekerengu Road	A	A	A	ISch	
33	19/04/98	O31 575745	Marlborough	Mt Fyffe	A, B	B	B	ISch	
34	23/04/98	J37 653952	S Canterbury	Orari-River Road (Mt Peel)	A	A	A	ISch	
35	23/04/98	J37 685005	S Canterbury	Peel Forest Park, Deer Spur Track, 5 km NW of tarn	A	A	A	ISch	
39	23/04/98	J37 684010	S-Canterbury	Peel Forest Park, Deer Spur Track	A	A	A	ISch	
43	25/04/98	G41 176792	Central Otago	Clutha valley near Crippletown	A	-	A	ISch	
58	13/05/98	Y18 565710	Gisborne	Makorori beach, 10 Min N of Gisborne	A	B	D	Jenkins	
64	17/05/98	H36 763136	S-Canterbury	Mt Cook National Park: Red Tarn	A-E	-	seedling (garden)	ISch	
65	31/05/98	N29 943313	Nelson	Mt Robert Skifield Road (Lake Rotoiti)	A, B, C	A	A	ISch	
66	31/5/98	N29 022220	Nelson	Wairau River Valley, Rainbow Ski Area	A, B, C, D	A	-	ISch	
67	31/05/98	???	Marlborough	Wairau River mainroad Nelson -Blenheim	A-F	A, B	B	ISch	
68	31/05/98	O29 427366	Marlborough	Waihopai River, end of gravel road	A-E	A	A	ISch	
70	18/01/98	R27 624094	Wellington	Te Korohiwa Rocks, Titahi Bay	A	A	A	Breitw.	2072
71	17/01/98	Z14 992753	Gisborne	Easte Cape at East Cape Lighthouse	A	A	-	Breitw.	2069
72	13/01/98	Y19 338343	Gisborne	North of Mahiha (North of Mahanga)	A	A	A	Breitw.	2067
75	09/09/98	K33 913111	Westland	Otira Valley, Southern Alps	-	seedling A	-	ISch	
81	20/1/98	R28 700728	Wellington	Turakirae Head, Cook Strait	A	A	A	ADW	
82	31/1/98	E43 516241	N Southland	Mt Eyre	A	A	A (2)	RMcK	

DNA Extraction

Table 1 continued

83	31/1/98	E43 49_23_	N-Southland	Mt Eyre	B	A, B	A, B	RMcK	
92	18/10/98	L34 360 735	Canterbury	Mt Oxford, East Brand, Coopers Creek	A	A	A	ISch	
94	26/4/94		Wellington	Lyall Bay	?	B	-	?	?
95	?/?/96		Canterbury	Poverty Flat, Mt White Road	?	B	-	M. Dawson	G302/96
98	23/4/97		Southland	Catlins State Forest Park	?	A (2x)	A	?	244/97
99	?/?/96		N-Canterbury	near St. James Walkway, Lewis Pass	?	A-B	-	M. Dawson	329/96
101	29/10/98	M24 857 773	Nelson	Cape Farewell		seedling	-	Garnock-Jones	2343
104	12/11/98	N1 163 505	North Cape	Cape Reinga, Coastal WW, cliff edge	A-I	A	A, B	ISch	
105	12/11/98	N1 163 475	North Cape	Te Werahi Beach	A-O	A	A, B, C, D	ISch	
106	12/11/98	N1 343 527	North Cape	Hooper Point	A-E	A (2x)	A	ISch	
107	12/11/98	N1 343 515	North Cape	Spirits Bay	A-I	B	A, B	ISch	
108	12/11/98	N03 188 005	N-Auckland	Ninety Mile Beach, Hukatere, Fire Lookout	A-D	A	A	ISch	
110	14/11/98	M28 701 602	Nelson	Mt Owen, Bulmer Lake	A	seedling	-	D. Glenny	7504
112	19/11/98	P20 048 103	Taranaki	Stratford Mt House ski field (Mt Egmont)	A	A	A	Weiss	
114	29/11/98	H46 625 245	S-Otago	3 km to Kaka Point (Nugget Point)	A-B	A	A, B	ISch	
115	29/11/98	I44 177 867	Otago	8.7 km to Dunedin, Kilmog Hill, HW1	A-C	A	A	ISch	
116	30/11/98	H46 545 157	S-Otago	roadside between Balclutha a. Owaka	A-C		A	ISch	
117	1/12/98	G47 319 942	Southland	Cathedral Cave Car Park	H, I	B, J	A, B, H, I	ISch	
118	1/12/98	G47 321 938	Southland	Cathedral Cave WW	A	B (seedl.)	-	ISch	
124	5/1/99	H43 74_ 21_	Otago	Rock + Pillar Range near McPhee's Rock	A-D	E (seedl.)	-	Garnock-Jones	2359

DNA Extraction

Table 1 continued

133	18/1/99	C44 732 806	Southland	Mt Burns	A	A	A	D. Glenny	7696
139	14/2/99	P27 902 925	Marlborough	Queen Charlotte Drive, Grove Arm	A-C	B	B	ISch	
141	14/2/99	P27 892 810	Marlborough	Parara Wetland, HW1	A-B	B	A, B	ISch	
143	14/2/99	P28 970 569	Marlborough	Weld Pass	A-F		A, B, C	ISch	
147	9/2/99	T20 165 463	Wellington	Ruapehu, Rangipo Desert	A		-	ADW	99120
149	8/3/99	O29 69_25_	Marlborough	Isis Stream (Awatere River)	A-J	X	A, B, C, G, H, I, J	ISch	
153	11/12/98	M27 743 093	NW Nelson	Near Mt Mytton	A, B	A	A, B	?	568-9/98
154	13/12/98	M24 873 776	Nelson	Puponga Farm, Farewell Spit	A		A	K. A. Ford	570/98
155	31/12/98	I41 027 806	Otago	Dansey's Pass	A, B		A, B	K. A. Ford	
158	28/04/99	N02 11- 55-	N-Auckland	Surville Cliffs	A		A	R. Smith	
159	28/04/99		Marlborough	Ben More	A		A	R. Smith	
160	18/01/99	L31 455 603	Westland	Blue-Grey River neat Mt Boscowen	A	A	A	K. A. Ford	
Cassinia aculeata			Tasmania	Mt Hartz National Park	A	A	-	JW	94115
Ozothamnus rodwayi			Tasmania	Mt Field National Park	A			JW	94155

DNA Extraction

Appendix 5

Colour characters

Table 1 Exudate colours (gradient green to yellow); character numbers 7, 33, 36, 39, 42.

Character states	Nickerson Color Fan distributed by American Horticulture Society, Published by Munsell Color Co. incorporated, 1957			
1	2.5	GY	9/8	brilliant yellow green
1	2.5	GY	8/9	brilliant yellow green
2	10	Y	9/9	brilliant greenish yellow
2	10	Y	8/11	strong greenish yellow
3	7.5	Y	9/8	brilliant greenish yellow
3	7.5	Y	8/12	vivid greenish yellow
4	5	Y	9/9	brilliant yellow
4	5	Y	8/12	vivid yellow
5	2.5	Y	9/9	brilliant yellow
5	2.5	Y	8/12	vivid yellow
6	7.5	YR	7/11(2.5Y8/12)	moderate orange yellow
7	10	YR	8/10	moderate orange yellow
7	10	YR	7/10	strong orange yellow
7	7.5	YR	7/11	strong orange yellow

Table 2 Exudate colours (depth); character numbers 8, 34, 37, 40, 43.

Character states	Nickerson Color Fan distributed by American Horticulture Society, Published by Munsell Color Co. incorporated, 1957			
1	2.5	GY	9/8	brilliant yellow green
1	2.5	Y	9/9	brilliant yellow
1	5	Y	9/9	brilliant yellow
1	7.5	Y	9/8	brilliant greenish yellow
1	10	Y	9/9	brilliant greenish yellow
2	2.5	GY	8/9	brilliant yellow green
2	2.5	Y	8/12	vivid yellow
2	5	Y	8/12	vivid yellow
2	7.5	Y	8/12	vivid greenish yellow
2	10	Y	8/11	strong greenish yellow
2	10	YR	8/10	moderate orange yellow
3	7.5	YR	7/11(2.5Y8/12)	moderate orange yellow
3	7.5	YR	7/11	strong orange yellow
3	10	YR	7/10	strong orange yellow

Table 3 Leaf colour abaxial surface (gradient from green to yellow); character numbers 15, 16.

Character states	Nickerson Color Fan distributed by American Horticulture Society, Published by Munsell Color Co. incorporated, 1957			
1	white (7.5 GY 9/4)			white
1	7.5	GY	9/4	light yellow green
1	7.5	GY	8/7	brilliant yellow green
1	7.5	GY	7/9	strong yellow green
2	white (2.5GY 9/8)			white/yellow
2	2.5	GY	9/8	brilliant yellow green
2	2.5	GY	8/9	brilliant yellow green
3	10	Y	9/9	brilliant greenish yellow
3	10	Y	8/11	strong greenish yellow
3	10	Y	7/9	strong greenish yellow
3	10	Y	6/7	dark greenish yellow
4	7.5	Y	9/8	brilliant greenish yellow
4	7.5	Y	8/12	vivid greenish yellow
4	7.5	Y	7/9	strong greenish yellow
4	7.5	Y	6/7	dark greenish yellow
5	5	Y	9/9	brilliant yellow
5	5	Y	8/12	vivid yellow
5	5	Y	7/10	strong yellow
5	5	Y	6/7	dark yellow
6	2.5	Y	9/9	brilliant yellow
6	2.5	Y	8/12	vivid yellow
6	2.5	Y	7/10	strong yellow
6	2.5	Y	6/8	deep yellow
7	10	YR	7/10	strong orange yellow

Table 4 Leaf colour abaxial surface (depth); character numbers 17, 18.

Character states	Nickerson Color Fan distributed by American Horticulture Society, Published by Munsell Color Co. incorporated, 1957			
1	white (7.5 GY 9/4)			white
1	white (2.5GY 9/8)			white/yellow
2	7.5	GY	9/4	light yellow green
2	2.5	GY	9/8	brilliant yellow green
2	10	Y	9/9	brilliant greenish yellow
2	7.5	Y	9/8	brilliant greenish yellow
2	5	Y	9/9	brilliant yellow
2	2.5	Y	9/9	brilliant yellow
3	7.5	GY	8/7	brilliant yellow green
3	2.5	GY	8/9	brilliant yellow green
3	10	Y	8/11	strong greenish yellow
3	7.5	Y	8/12	vivid greenish yellow
3	5	Y	8/12	vivid yellow
3	2.5	Y	8/12	vivid yellow
4	7.5	GY	7/9	strong yellow green
4	10	Y	7/9	strong greenish yellow
4	7.5	Y	7/9	strong greenish yellow
4	5	Y	7/10	strong yellow
4	2.5	Y	7/10	strong yellow
4	10	YR	7/10	strong orange yellow
5	10	Y	6/7	dark greenish yellow
5	7.5	Y	6/7	dark greenish yellow
5	5	Y	6/7	dark yellow
5	2.5	Y	6/8	deep yellow

Table 5 Leaf colour adaxial surface (gradient from green to yellow); character numbers 11, 12.

Character states	Nickerson Color Fan distributed by American Horticulture Society, Published by Munsell Color Co. incorporated, 1957			
1	10	GY	4/5	dark yellowish green
1	10	GY	5/8	strong yellowish green
1	10	GY	6/9	strong yellowish green
2	7.5	GY	4/4	moderate olive green
2	7.5	GY	5/7	moderate yellow green
2	7.5	GY	6/8	strong yellow green
2	7.5	GY	7/9	strong yellow green
2	7.5	GY	8/7	brilliant yellow green
2	7.5	GY	9/4	light yellow green
3	5	GY	3/2	grayish olive green
3	5	GY	4/3	moderate olive green
3	5	GY	5/6	moderate yellow green
3	5	GY	6/8	strong yellow green
3	5	GY	7/10	strong yellow green
3	5	GY	8/8	brilliant yellow green
4	2.5	GY	4/3	moderate olive green
4	2.5	GY	5/5	moderate yellow green
4	2.5	GY	6/8	strong yellow green
4	2.5	GY	7/10	strong yellow green
4	2.5	GY	8/9	brilliant yellow green
5	10	Y	4/3	moderate olive
5	10	Y	5/5	light olive
5	10	Y	6/7	dark greenish yellow
5	10	Y	7/9	strong greenish yellow
6	7.5	Y	4/3	moderate olive
6	7.5	Y	5/5	light olive
6	7.5	Y	6/7	dark greenish yellow
6	7.5	Y	7/9	strong greenish yellow
7	5	Y	4/3	moderate olive
7	5	Y	5/5	light olive
7	5	Y	6/7	dark yellow
8	2.5	Y	4/4	moderate olive brown
9	10	YR	4/4	moderate yellowish brown
9	10	YR	5/6	strong yellowish brown
10	7.5	YR	3/2	grayish brown

Table 6 Leaf colour adaxial surface (depth); character numbers 13, 14.

Character states	Nickerson Color Fan distributed by American Horticulture Society, Published by Munsell Color Co. incorporated, 1957			
1	7.5	GY	9/4	light yellow green
2	2.5	GY	8/9	brilliant yellow green
2	5	GY	8/8	brilliant yellow green
2	7.5	GY	8/7	brilliant yellow green
3	2.5	GY	7/10	strong yellow green
3	5	GY	7/10	strong yellow green
3	7.5	GY	7/9	strong yellow green
3	7.5	Y	7/9	strong greenish yellow
3	10	Y	7/9	strong greenish yellow
4	5	GY	6/8	strong yellow green
4	7.5	GY	6/8	strong yellow green
4	10	GY	6/9	strong yellowish green
4	2.5	GY	6/8	strong yellow green
4	5	Y	6/7	dark yellow
4	7.5	Y	6/7	dark greenish yellow
4	10	Y	6/7	dark greenish yellow
5	2.5	GY	5/5	moderate yellow green
5	5	GY	5/6	moderate yellow green
5	7.5	GY	5/7	moderate yellow green
5	10	GY	5/8	strong yellowish green
5	10	YR	5/6	strong yellowish brown
5	5	Y	5/5	light olive
5	7.5	Y	5/5	light olive
5	10	Y	5/5	light olive
6	2.5	GY	4/3	moderate olive green
6	5	GY	4/3	moderate olive green
6	7.5	GY	4/4	moderate olive green
6	10	GY	4/5	dark yellowish green
6	2.5	Y	4/4	moderate olive brown
6	10	YR	4/4	moderate yellowish brown
6	5	Y	4/3	moderate olive
6	7.5	Y	4/3	moderate olive
6	10	Y	4/3	moderate olive
7	5	GY	3/2	grayish olive green
7	7.5	YR	3/2	grayish brown

Appendix 6

Correlations for colour characters (see table) created by S-Plus

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.000	0.864	0.480	0.210	0.036	0.020	0.688	0.696	0.569	0.512	0.575	0.485	0.768	0.692	0.593	0.516	0.756	0.686
2	0.864	1.000	0.422	0.235	0.086	0.059	0.729	0.743	0.636	0.527	0.537	0.476	0.751	0.735	0.527	0.465	0.732	0.723
3	0.480	0.422	1.000	0.764	0.288	0.133	0.557	0.510	0.580	0.579	0.320	0.323	0.435	0.479	0.290	0.291	0.418	0.461
4	0.210	0.235	0.764	1.000	0.321	0.108	0.410	0.360	0.533	0.511	0.173	0.232	0.244	0.360	0.125	0.166	0.239	0.354
5	0.036	0.086	0.288	0.321	1.000	0.733	0.028	0.012	0.226	0.180	0.169	0.109	0.023	0.029	0.182	0.104	0.024	0.025
6	0.020	0.059	0.133	0.108	0.733	1.000	0.066	0.053	0.119	0.106	0.090	0.087	0.045	0.084	0.134	0.138	0.039	0.082
7	0.688	0.729	0.557	0.410	0.028	0.066	1.000	0.880	0.718	0.594	0.536	0.462	0.644	0.640	0.473	0.394	0.629	0.623
8	0.696	0.743	0.510	0.360	0.012	0.053	0.880	1.000	0.710	0.653	0.512	0.428	0.660	0.660	0.537	0.448	0.659	0.671
9	0.569	0.636	0.580	0.533	0.226	0.119	0.718	0.710	1.000	0.771	0.451	0.460	0.589	0.660	0.416	0.414	0.576	0.646
10	0.512	0.527	0.579	0.511	0.180	0.106	0.594	0.653	0.771	1.000	0.298	0.315	0.456	0.532	0.305	0.315	0.474	0.554
11	0.575	0.537	0.320	0.173	0.169	0.090	0.536	0.512	0.451	0.298	1.000	0.888	0.566	0.564	0.938	0.824	0.573	0.556
12	0.485	0.476	0.323	0.232	0.109	0.087	0.462	0.428	0.460	0.315	0.888	1.000	0.470	0.563	0.816	0.918	0.481	0.557
13	0.768	0.751	0.435	0.244	0.023	0.045	0.644	0.660	0.589	0.456	0.566	0.470	1.000	0.766	0.559	0.451	0.984	0.752
14	0.692	0.735	0.479	0.360	0.029	0.084	0.640	0.660	0.660	0.532	0.564	0.563	0.766	1.000	0.557	0.547	0.755	0.983
15	0.593	0.527	0.290	0.125	0.182	0.134	0.473	0.537	0.416	0.305	0.938	0.816	0.559	0.557	1.000	0.889	0.577	0.574
16	0.516	0.465	0.291	0.166	0.104	0.138	0.394	0.448	0.414	0.315	0.824	0.918	0.451	0.547	0.889	1.000	0.473	0.567
17	0.756	0.732	0.418	0.239	0.024	0.039	0.629	0.659	0.576	0.474	0.573	0.481	0.984	0.755	0.577	0.473	1.000	0.762
18	0.686	0.723	0.461	0.354	0.025	0.082	0.623	0.671	0.646	0.554	0.556	0.557	0.752	0.983	0.574	0.567	0.762	1.000

indicates correlation ≥0.66

Colour characters

	Colour Character
1	branchlet exudate colour gradient from green to yellow
2	branchlet exudate colour depth
3	colour of adaxial surface of young leaf gradient from green to yellow
4	colour of adaxial surface of mature leaf gradient from green to yellow
5	colour of adaxial surface of young leaf depth
6	colour of adaxial surface of mature leaf depth
7	colour of abaxial surface of young leaf gradient from green to yellow
8	colour of abaxial surface of mature leaf gradient from green to yellow
9	colour of abaxial surface of young leaf depth
10	colour of abaxial surface of mature leaf depth
11	exudate colour (gradient green to yellow) on adaxial surface of lamina of young leaf
12	exudate colour (depth) on adaxial surface of lamina of young leaf
13	exudate colour (gradient green to yellow) on abaxial surface of lamina of young leaf
14	exudate colour (depth) on abaxial surface of lamina of young leaf
15	exudate colour (gradient green to yellow) on adaxial surface of lamina of mature leaf
16	exudate colour (depth) on adaxial surface of lamina of mature leaf
17	exudate colour (gradient green to yellow) on abaxial surface of lamina of mature leaf
18	exudate colour (depth) on abaxial surface of lamina of mature leaf

Characters excluded from analysis

Appendix 7

Table 1 Statistics of the absolute values of the quantitative multi-state characters for 145 specimens from 82 different populations and 10 different groups. Including highest (max), lowest (min) and average (mean), mean value of each character, and the highest, lowest and average standard deviation (stdev) and standard error (se) of the mean of the ten measurements for each character.

number	character	max	min	mean	stdev max	stdev min	stdev mean	se max	se min	se mean
3	branchlet angle (°)	64.80	20.71	37.14	19.41	3.43	8.53	0.52	0.07	0.23
4	branchlet diameter (mm)	3.06	0.78	1.41	3.73	0.10	0.26	1.22	0.06	0.18
10	branchlet spacing (number/5 cm)	21.20	3.38	8.96	6.65	0.58	2.30	0.79	0.09	0.27
19	lamina orientation (°)	89.00	36.00	58.55	20.49	4.74	11.32	0.40	0.08	0.20
24	lamina length (mm)	13.18	2.30	5.86	2.70	0.21	0.86	0.28	0.06	0.15
25	lamina width (mm)	4.76	0.97	2.35	1.45	0.08	0.35	0.44	0.06	0.15
26	ratio (lamina length/ lamina width)	4.60	1.66	2.57	0.94	0.07	0.27	0.34	0.03	0.10
47	leaf spacing (number per cm)	21.00	3.90	9.34	5.36	0.63	2.08	0.46	0.08	0.23
49	number of capitula per panicle	301.83	5.50	54.27	247.61	0.58	17.95	0.82	0.05	0.28
50	panicle diameter (mm)	70.33	7.75	23.59	15.48	0.00	4.01	0.38	0.00	0.17
51	diameter of panicle / (number of capitula per panicle x body width of capitulum)	2.45	0.17	0.68	0.49	0.01	0.12	0.55	0.03	0.18
53	pedicel (mm)	5.83	0.28	1.63	2.02	0.15	0.67	1.14	0.17	0.46
54	capitulum top width (diameter) (mm)	4.79	1.16	2.72	0.56	0.10	0.25	0.21	0.03	0.10
55	capitulum length (mm)	8.83	3.83	5.50	0.61	0.11	0.28	0.13	0.02	0.05
56	involucre length (mm)	6.71	3.26	4.61	0.71	0.11	0.27	0.18	0.02	0.06
57	capitulum body width (mm)	3.20	0.96	1.89	0.37	0.05	0.14	0.21	0.03	0.07
58	ratio of body width (involucre) to capitulum length	0.49	0.19	0.34	0.08	0.01	0.03	0.27	0.04	0.08
59	ratio of top width to body width	2.00	1.08	1.46	0.47	0.05	0.15	0.31	0.03	0.11
60	floret number per capitulum	19.90	4.40	10.22	3.33	0.32	1.03	0.28	0.03	0.11
61	corolla tube length (mm)	4.79	1.86	2.70	0.55	0.04	0.16	0.18	0.02	0.06
62	corolla lobe length (mm)	0.96	0.39	0.59	0.10	0.02	0.05	0.23	0.03	0.09
63	corolla lobe width (mm)	0.05	0.21	0.31	0.13	0.01	0.04	0.37	0.05	0.11
64	corolla circumference (mm)	1.60	0.59	0.93	0.32	0.06	0.14	0.40	0.06	0.16
65	style length (without stigmatic lobe) (mm)	4.67	1.64	2.42	0.39	0.03	0.17	0.17	0.01	0.07
66	stigmatic lobe length (mm)	1.19	0.38	0.66	0.23	0.03	0.08	0.35	0.04	0.12
71	outer involucral bracts number	20.00	5.90	10.78	2.58	0.47	1.09	0.24	0.03	0.10
72	inner involucral bracts length (mm)	6.22	2.82	4.05	0.46	0.06	0.24	0.12	0.01	0.06
73	inner involucral bracts width (mm)	1.61	0.40	0.84	2.81	0.07	0.17	1.74	0.07	0.19
74	ratio of inner involucral bracts length to width	11.12	3.43	5.14	2.85	0.35	1.10	0.53	0.07	0.21
77	inner involucral bracts number	15.40	4.60	7.77	1.93	0.00	0.86	0.26	0.00	0.11
78	total number of inv. bracts	30.40	12.10	18.52	2.61	0.32	1.30	0.18	0.02	0.07
79	number of receptacle scales	26.90	0.00	7.32	3.45	0.00	1.23	0.77	0.03	0.22
80	receptacle scales length (mm)	6.88	2.81	4.11	1.18	0.08	0.27	0.30	0.02	0.07
81	receptacle scales width (mm)	0.82	0.28	0.45	0.23	0.05	0.11	0.41	0.09	0.24
82	ratio of receptacle scales length to width	16.03	5.78	9.93	7.27	0.69	2.61	0.77	0.10	0.26
83	total number of white tipped appendages (mm)	34.20	6.50	15.09	3.36	0.47	1.46	0.22	0.02	0.10
84	pappus length (mm)	5.02	2.01	3.02	0.31	0.04	0.14	0.09	0.01	0.05
86	achenes length (mm)	1.81	0.59	1.13	0.31	0.00	0.13	0.39	0.00	0.12
87	achenes width (mm)	0.61	0.27	0.45	0.13	0.01	0.05	0.34	0.03	0.12
88	ratio of achenes length to width (mm)	3.60	1.73	2.51	0.94	0.11	0.31	0.30	0.04	0.12

Table 2 Statistics of the absolute values of the quantitative multi-state characters for 12 specimens from the homogeneous Cass Population. Including highest (max), lowest (min) and average (mean), mean value of each character, and the highest, lowest and average standard deviation (stdev) and standard error (se) of the mean of the ten measurements for each character.

number	character	max	min	mean	stdev max	stdev min	stdev mean	se max	se min	se mean
3	branchlet angle (°)	45.00	27.50	33.13	10.07	4.83	7.25	0.37	0.14	0.22
4	branchlet diameter (mm)	1.33	1.04	1.20	0.28	0.11	0.17	0.25	0.10	0.14
10	branchlet spacing (number/5 cm)	12.90	9.75	11.03	3.78	1.33	2.55	0.32	0.12	0.23
19	lamina orientation (°)	49.00	41.00	45.54	13.55	7.82	10.57	0.33	0.17	0.23
24	lamina length (mm)	5.08	3.76	4.59	1.22	0.23	0.60	0.25	0.05	0.13
25	lamina width (mm)	2.06	1.30	1.64	0.29	0.06	0.20	0.18	0.04	0.12
26	ratio (lamina length/ lamina width)	3.69	2.40	2.83	0.59	0.16	0.30	0.18	0.06	0.10
47	leaf spacing (number per cm)	12.00	7.10	9.72	3.61	1.52	2.41	0.35	0.17	0.25
49	number of capitula per panicle	54.00	15.00	34.60	19.78	1.00	10.56	0.51	0.07	0.30
50	panicle diameter (mm)	25.00	9.67	17.47	6.18	2.19	3.75	0.31	0.12	0.22
51	diameter of panicle / (number of capitula per panicle x body width of capitulum)	0.39	0.24	0.30						
53	pedicel (mm)	2.31	0.84	1.39	0.88	0.28	0.45	0.53	0.14	0.35
54	capitulum top width (diameter) (mm)	3.12	2.38	2.62	0.27	0.12	0.17	0.10	0.05	0.07
55	capitulum length (mm)	5.04	4.47	4.76	0.32	0.12	0.21	0.06	0.02	0.04
56	involucre length (mm)	4.14	3.46	3.72	0.48	0.12	0.23	0.12	0.03	0.06
57	capitulum body width (mm)	1.96	1.51	1.71	0.16	0.07	0.11	0.09	0.04	0.07
58	ratio of body width (involucre) to capitulum length	0.40	0.33	0.36	0.03	0.01	0.02	0.09	0.03	0.07
59	ratio of top width to body width	1.65	1.40	1.54	0.14	0.06	0.10	0.09	0.03	0.06
60	floret number per capitulum	12.30	6.30	9.07	1.58	0.00	1.07	0.21	0.00	0.12
61	corolla tube length (mm)	2.48	2.04	2.34	0.15	0.03	0.10	0.07	0.01	0.04
62	corolla lobe length (mm)	0.63	0.52	0.57	0.07	0.03	0.04	0.11	0.05	0.07
63	corolla lobe width (mm)	0.36	0.27	0.30	0.04	0.02	0.03	0.13	0.05	0.09
64	corolla circumference (mm)	1.76	0.72	0.94	0.16	0.06	0.11	0.18	0.05	0.12
65	style length (without stigmatic lobe) (mm)	2.10	0.65	1.74	0.20	0.09	0.13	0.17	0.05	0.08
66	stigmatic lobe length (mm)	0.87	0.62	0.73	0.11	0.04	0.07	0.15	0.05	0.10
71	outer involucral bracts number	11.20	7.80	9.36	1.26	0.63	1.03	0.15	0.08	0.11
72	inner involucral bracts length (mm)	3.86	3.31	3.54	0.32	0.11	0.20	0.09	0.03	0.06
73	inner involucral bracts width (mm)	1.02	0.73	0.85	0.16	0.10	0.13	0.21	0.12	0.16
74	ratio of inner involucral bracts length to width	5.01	3.62	4.32	1.31	0.61	0.88	0.26	0.16	0.20
77	inner involucral bracts number	8.20	6.20	7.40	0.99	0.48	0.72	0.12	0.07	0.10
78	total number of inv. bracts	19.40	15.20	16.82	1.62	0.67	1.07	0.10	0.04	0.06
79	number of receptacle scales	14.40	4.30	8.60	2.50	0.67	1.52	0.25	0.13	0.18
80	receptacle scales length (mm)	3.79	3.30	3.53	0.36	0.14	0.25	0.10	0.04	0.07
81	receptacle scales width (mm)	0.56	0.40	0.45	0.14	0.06	0.11	0.33	0.13	0.24
82	ratio of receptacle scales length to width	9.75	6.85	8.33	3.68	0.88	2.11	0.38	0.13	0.24
83	total number of white tipped appendages (mm)	21.90	11.00	16.05	2.72	1.06	1.69	0.15	0.07	0.11
84	pappus length (mm)	3.03	2.52	2.76	0.20	0.06	0.13	0.07	0.02	0.05
86	achenes length (mm)	1.34	1.01	1.13	0.21	0.05	0.13	0.20	0.04	0.12
87	achenes width (mm)	0.49	0.37	0.42	0.07	0.02	0.05	0.18	0.05	0.12
88	ratio of achenes length to width (mm)	3.12	2.22	2.71	0.61	0.10	0.29	0.20	0.03	0.11

Table 3 Standard deviation (stdev) and standard error (se) of the mean values of the quantitative multi-state characters for 12 specimens from the homogeneous Cass Population

number	character	stdev	se
3	branchlet angle (°)	4.47	0.13
4	branchlet diameter (mm)	0.10	0.08
10	branchlet spacing (number/5 cm)	1.17	0.11
19	lamina orientation (°)	2.73	0.06
24	lamina length (mm)	0.40	0.09
25	lamina width (mm)	0.18	0.11
26	ratio (lamina length/ lamina width)	0.36	0.13
47	leaf spacing (number per cm)	1.33	0.14
49	number of capitula per panicle	10.75	0.31
50	panicle diameter (mm)	4.68	0.27
51	diameter of panicle / (number of capitula per panicle x body width of capitulum)	0.05	0.17
53	pedicel (mm)	0.56	0.40
54	capitulum top width (diameter) (mm)	0.20	0.08
55	capitulum length (mm)	0.24	0.05
56	involucre length (mm)	0.25	0.07
57	capitulum body width (mm)	0.15	0.09
58	ratio of body width (involucre) to capitulum length	0.02	0.06
59	ratio of top width to body width	0.08	0.05
60	floret number per capitulum	1.67	0.18
61	corolla tube length (mm)	0.14	0.06
62	corolla lobe length (mm)	0.03	0.06
63	corolla lobe width (mm)	0.03	0.09
64	corolla circumference (mm)	0.28	0.30
65	style length (without stigmatic lobe) (mm)	0.37	0.22
66	stigmatic lobe length (mm)	0.07	0.10
71	outer involucral bracts number	1.08	0.12
72	inner involucral bracts length (mm)	0.16	0.04
73	inner involucral bracts width (mm)	0.09	0.11
74	ratio of inner involucral bracts length to width	0.47	0.11
77	inner involucral bracts number	0.63	0.08
78	total number of inv. bracts	1.50	0.09
79	number of receptacle scales	3.70	0.43
80	receptacle scales length (mm)	0.17	0.05
81	receptacle scales width (mm)	0.05	0.11
82	ratio of receptacle scales length to width	1.10	0.13
83	total number of white tipped appendages (mm)	4.11	0.26
84	pappus length (mm)	0.14	0.05
86	achenes length (mm)	0.11	0.09
87	achenes width (mm)	0.04	0.08
88	ratio of achenes length to width (mm)	0.26	0.10

Table 4 Statistics of the absolute values of the quantitative multi-state microcharacters (MC) based on 23 specimens from 23 different populations and 10 different groups. Shown are highest (max), lowest (min) and average (mean), mean value of each character, and the highest, lowest and average standard deviation (stdev) and standard error (se) of the mean of the ten or 30 (*) measurements for each character.

number		character	max	min	mean	stdev max	stdev min	stdev mean	se max	se min	se mean
Pappus characters	MC1	pappus width at tip (µm)*	88.50	42.50	61.81	17.49	6.88	10.71	0.26	0.12	0.18
	MC2	number of apical cells*	5.20	3.00	4.05	0.97	0.42	0.64	0.24	0.10	0.16
	MC3	width of apical cells (µm)*	34.58	15.88	26.45	5.68	1.58	3.27	0.17	0.07	0.12
	MC4	width at widest part (µm)*	101.75	65.25	80.69	25.29	7.86	14.89	0.29	0.09	0.19
	MC5	width at centre (µm)*	27.25	18.75	22.90	4.83	1.58	3.10	0.19	0.07	0.14
	MC6	length of barbellae (µm)*	58.42	18.58	33.33	45.72	6.11	12.47	1.21	0.21	0.38
	MC7	distance between barbellae and axis (µm)*	22.42	10.00	14.89	7.80	2.56	4.59	0.42	0.21	0.31
MC10		anther insertion point (mm)	1.40	0.53	0.95	0.17	0.04	0.10	0.28	0.05	0.10
MC11		corolla tube length (mm)	3.94	2.14	2.78	0.26	0.06	0.15	0.08	0.02	0.05
MC12		corolla tube length : anther insertion point	4.44	2.42	2.99	1.06	0.05	0.24	0.24	0.02	0.08
MC13		anther connective base length (µm)	34.00	15.30	23.74	5.01	1.25	2.34	0.15	0.06	0.10
MC14		anther basal appendage length (µm)	53.70	25.90	36.09	11.83	2.10	3.68	0.31	0.06	0.10
MC15		anther connective base length : anther basal appendage length	0.88	0.40	0.67	0.24	0.03	0.07	0.28	0.04	0.10

Appendix 8

Similarity matrices

Similarity matrices from Data Set 1, Data Set 2, Data Set 3 with Gower's coefficient are on included disk.

Similarity matrix from Data Set 4 (reduced data set) with Gower's coefficient

Similarity matrix from Data Set 5 (reduced data set plus additional microcharacters) with Gower's coefficient

Similarity matrix from Data Set 6 (23 OTUs of which microcharacters are available) with Gower's coefficient

Similarity matrix from Data Set 7 (microcharacters only) with Gower's coefficient

Similarity matrix from Data Set 8 (vegetative characters only) with Gower's coefficient

Similarity matrix from Data Set 9 (floral characters only) with Gower's coefficient

Similarity matrix from AFLP data with Jaccard's coefficient

Similarity matrix from a morphological data set including only the 23 OTUs included in the molecular study with Gower's coefficient

Similarity matrix from morphological data including only the 12 representatives of the Cass population (P1-P12) with Gower's coefficient

Similarity matrix from AFLP data including only the 12 representatives of the Cass population (P1-P12) with Jaccard's coefficient

Similarity matrix from Data Set 4 (reduced data set) with Gower's coefficient

	158/f	CHR	159A	65A/f	66B/d	67A/f	11A/f	33B/f	64A/f	118B/	17A/f	8G/fc	7C/fc	5A/d	77A/ff	34A/f	43A/d	139B/	58B/f	70/fc	112A	148A	9A/fc	13A/f	13B/f	4X/ff	15A/f	35A/f	134A	105A	105A/	106A/	108A
158/fc	1.00																																
CHR	0.81	1.00																															
159A/d	0.70	0.79	1.00																														
65A/fc	0.66	0.75	0.76	1.00																													
66B/d	0.66	0.71	0.70	0.71	1.00																												
67A/fc	0.75	0.73	0.67	0.77	0.78	1.00																											
11A/fc	0.74	0.68	0.71	0.75	0.77	0.80	1.00																										
33B/fc	0.73	0.71	0.77	0.76	0.74	0.76	0.75	1.00																									
64A/fc	0.68	0.69	0.61	0.67	0.75	0.78	0.77	0.63	1.00																								
118B/fc	0.61	0.68	0.60	0.69	0.74	0.77	0.76	0.64	0.81	1.00																							
17A/fc	0.65	0.68	0.62	0.69	0.73	0.79	0.78	0.64	0.82	0.86	1.00																						
8G/fc	0.58	0.63	0.56	0.61	0.66	0.63	0.69	0.54	0.70	0.67	0.67	1.00																					
7C/fc	0.63	0.63	0.54	0.58	0.60	0.62	0.64	0.52	0.73	0.64	0.67	0.84	1.00																				
5A/d	0.54	0.64	0.53	0.59	0.67	0.63	0.66	0.53	0.69	0.69	0.67	0.83	0.81	1.00																			
77A/ff	0.67	0.67	0.61	0.64	0.70	0.73	0.70	0.60	0.77	0.75	0.76	0.74	0.76	0.75	1.00																		
34A/fc	0.62	0.63	0.58	0.63	0.69	0.69	0.67	0.56	0.76	0.69	0.69	0.81	0.77	0.80	0.77	1.00																	
43A/d	0.51	0.59	0.46	0.58	0.67	0.65	0.65	0.56	0.69	0.66	0.63	0.73	0.70	0.80	0.74	0.79	1.00																
139B/fc	0.59	0.67	0.70	0.64	0.68	0.67	0.67	0.65	0.65	0.64	0.69	0.72	0.70	0.69	0.77	0.71	0.66	1.00															
58B/fc	0.72	0.67	0.68	0.65	0.66	0.65	0.67	0.61	0.69	0.60	0.62	0.77	0.78	0.72	0.73	0.74	0.70	0.77	1.00														
70/fc	0.56	0.68	0.74	0.67	0.67	0.67	0.67	0.67	0.65	0.61	0.64	0.73	0.70	0.69	0.69	0.69	0.67	0.84	0.80	1.00													
112A/fc	0.58	0.67	0.54	0.64	0.74	0.75	0.77	0.62	0.83	0.81	0.83	0.66	0.66	0.69	0.71	0.69	0.67	0.61	0.61	0.61	1.00												
148A/d	0.58	0.67	0.66	0.62	0.74	0.71	0.75	0.62	0.76	0.75	0.76	0.66	0.61	0.69	0.67	0.65	0.66	0.60	0.62	0.63	0.86	1.00											
9A/fc	0.64	0.75	0.72	0.72	0.74	0.76	0.79	0.70	0.82	0.77	0.79	0.74	0.67	0.70	0.72	0.72	0.69	0.74	0.72	0.75	0.77	0.77	1.00										
13A/fc	0.60	0.69	0.59	0.65	0.70	0.76	0.75	0.59	0.86	0.84	0.83	0.72	0.75	0.75	0.79	0.73	0.69	0.68	0.68	0.66	0.83	0.78	0.81	1.00									
13B/fc	0.61	0.70	0.59	0.65	0.70	0.75	0.77	0.60	0.78	0.77	0.78	0.73	0.70	0.71	0.73	0.71	0.67	0.64	0.65	0.64	0.78	0.74	0.82	0.83	1.00								
4X/ff	0.52	0.64	0.59	0.61	0.71	0.70	0.72	0.56	0.79	0.76	0.74	0.76	0.74	0.80	0.74	0.79	0.80	0.65	0.70	0.68	0.78	0.77	0.79	0.82	0.77	1.00							
15A/fc	0.57	0.65	0.56	0.63	0.69	0.71	0.71	0.57	0.81	0.75	0.77	0.77	0.76	0.80	0.77	0.82	0.80	0.66	0.71	0.68	0.75	0.71	0.78	0.84	0.78	0.85	1.00						
35A/fc	0.57	0.66	0.54	0.73	0.73	0.75	0.76	0.64	0.80	0.80	0.79	0.72	0.71	0.72	0.76	0.73	0.71	0.63	0.66	0.65	0.78	0.74	0.81	0.83	0.85	0.77	0.81	1.00					
134A/d	0.55	0.65	0.63	0.59	0.71	0.68	0.74	0.61	0.75	0.78	0.74	0.64	0.58	0.72	0.66	0.67	0.70	0.62	0.59	0.63	0.78	0.82	0.74	0.76	0.74	0.77	0.74	0.73	1.00				
105A/d	0.60	0.68	0.72	0.57	0.59	0.59	0.63	0.62	0.58	0.59	0.62	0.63	0.64	0.62	0.67	0.60	0.54	0.69	0.61	0.67	0.59	0.63	0.66	0.65	0.67	0.63	0.60	0.60	0.59	1.00			
105A/fc	0.72	0.71	0.77	0.63	0.64	0.68	0.63	0.69	0.59	0.63	0.67	0.54	0.56	0.53	0.63	0.51	0.49	0.67	0.61	0.69	0.60	0.58	0.68	0.61	0.67	0.57	0.56	0.61	0.59	0.81	1.00		
106A/fc	0.67	0.68	0.80	0.65	0.67	0.66	0.64	0.70	0.62	0.68	0.69	0.58	0.58	0.58	0.69	0.55	0.54	0.70	0.59	0.70	0.64	0.63	0.68	0.63	0.66	0.59	0.57	0.63	0.64	0.81	0.86	1.00	
108A/fc	0.68	0.62	0.62	0.58	0.65	0.72	0.71	0.62	0.67	0.73	0.74	0.61	0.62	0.62	0.71	0.58	0.59	0.62	0.58	0.60	0.72	0.68	0.68	0.73	0.74	0.66	0.65	0.70	0.68	0.68	0.80	0.80	1.00

Similarity matrix from Data Set 5 (reduced data set plus additional microcharacters) with Gower's coefficient

	158/fc	CHR	159A/d	65A/fc	66B/d	67A/fc	11A/fc	33B/fc	64A/fc	118B/f	17A/fc	8G/fc	7C/fc	5A/d	77A/ff	34A/fc	43A/d	139B/f	58B/fc	70/fc	112A/f	148A/d	9A/fc	13A/fc	4X/ff	15A/fc	35A/fc	105A/f	106A/f	108A/f
158/fc	1.00																													
CHR	0.80	1.00																												
159A/d	0.69	0.79	1.00																											
65A/fc	0.65	0.73	0.75	1.00																										
66B/d	0.65	0.72	0.69	0.69	1.00																									
67A/fc	0.75	0.71	0.66	0.76	0.78	1.00																								
11A/fc	0.70	0.69	0.69	0.75	0.76	0.81	1.00																							
33B/fc	0.66	0.72	0.76	0.77	0.73	0.77	0.74	1.00																						
64A/fc	0.69	0.69	0.61	0.67	0.76	0.77	0.77	0.62	1.00																					
118B/fc	0.64	0.67	0.58	0.68	0.73	0.76	0.74	0.62	0.82	1.00																				
17A/fc	0.64	0.69	0.61	0.68	0.73	0.79	0.76	0.63	0.80	0.83	1.00																			
8G/fc	0.52	0.62	0.56	0.64	0.65	0.61	0.69	0.57	0.66	0.63	0.62	1.00																		
7C/fc	0.59	0.62	0.54	0.61	0.59	0.61	0.65	0.55	0.69	0.63	0.64	0.84	1.00																	
5A/d	0.53	0.63	0.52	0.57	0.64	0.61	0.63	0.51	0.68	0.67	0.65	0.83	0.82	1.00																
77A/ff	0.64	0.67	0.59	0.66	0.68	0.71	0.70	0.62	0.74	0.71	0.73	0.75	0.77	0.74	1.00															
34A/fc	0.60	0.61	0.57	0.66	0.67	0.67	0.68	0.59	0.72	0.66	0.66	0.80	0.78	0.80	0.79	1.00														
43A/d	0.52	0.58	0.45	0.61	0.67	0.63	0.67	0.59	0.67	0.65	0.62	0.74	0.72	0.80	0.76	0.79	1.00													
139B/fc	0.56	0.67	0.69	0.66	0.66	0.66	0.68	0.65	0.63	0.62	0.67	0.72	0.72	0.68	0.77	0.73	0.68	1.00												
58B/fc	0.67	0.67	0.69	0.68	0.65	0.64	0.69	0.63	0.67	0.60	0.62	0.77	0.78	0.72	0.75	0.75	0.72	0.78	1.00											
70/fc	0.50	0.68	0.74	0.68	0.65	0.65	0.67	0.67	0.61	0.58	0.61	0.74	0.71	0.68	0.70	0.70	0.69	0.84	0.80	1.00										
112A/fc	0.61	0.68	0.55	0.66	0.74	0.75	0.77	0.64	0.80	0.78	0.82	0.64	0.65	0.67	0.72	0.69	0.67	0.62	0.61	0.60	1.00									
148A/d	0.60	0.67	0.66	0.64	0.74	0.70	0.75	0.63	0.76	0.74	0.74	0.64	0.62	0.67	0.67	0.65	0.66	0.60	0.63	0.61	0.84	1.00								
9A/fc	0.63	0.76	0.72	0.72	0.74	0.75	0.78	0.70	0.80	0.75	0.76	0.70	0.66	0.68	0.71	0.71	0.67	0.71	0.71	0.71	0.75	0.76	1.00							
13A/fc	0.61	0.70	0.60	0.67	0.70	0.76	0.77	0.60	0.82	0.82	0.82	0.70	0.74	0.74	0.79	0.72	0.69	0.69	0.68	0.65	0.81	0.77	0.79	1.00						
4X/ff	0.51	0.64	0.59	0.60	0.71	0.69	0.72	0.56	0.79	0.75	0.74	0.75	0.74	0.78	0.73	0.77	0.78	0.63	0.68	0.66	0.78	0.76	0.78	0.81	1.00					
15A/fc	0.57	0.65	0.56	0.66	0.70	0.70	0.72	0.60	0.78	0.73	0.75	0.75	0.75	0.79	0.76	0.81	0.80	0.68	0.71	0.68	0.76	0.70	0.77	0.83	0.85	1.00				
35A/fc	0.58	0.66	0.54	0.75	0.73	0.75	0.78	0.66	0.78	0.78	0.78	0.71	0.71	0.71	0.76	0.73	0.72	0.64	0.67	0.65	0.79	0.74	0.80	0.82	0.77	0.80	1.00			
105A/fc	0.65	0.72	0.79	0.63	0.64	0.68	0.64	0.69	0.58	0.61	0.66	0.56	0.57	0.52	0.64	0.53	0.52	0.68	0.62	0.69	0.59	0.59	0.67	0.62	0.57	0.58	0.62	1.00		
106A/fc	0.63	0.69	0.80	0.66	0.66	0.66	0.65	0.70	0.61	0.66	0.68	0.59	0.61	0.57	0.70	0.58	0.57	0.71	0.62	0.70	0.65	0.65	0.67	0.64	0.58	0.59	0.65	0.84	1.00	
108A/fc	0.68	0.63	0.63	0.58	0.65	0.72	0.72	0.62	0.66	0.73	0.74	0.60	0.60	0.61	0.71	0.58	0.58	0.61	0.56	0.59	0.71	0.67	0.68	0.72	0.66	0.65	0.70	0.79	0.79	1.00

Similarity matrix from Data Set 6 (23 OTUs of which microcharacters are available) with Gower's coefficient

	158/fc	65A/fc	11A/fc	33B/fc	64A/fc	118B/fc	17A/fc	8G/fc	7C/fc	77A/ff	34A/fc	43A/d	139B/fc	58B/fc	70/fc	112A/fc	148A/d	9A/fc	13A/fc	15A/fc	35A/fc	105A/fc	106A/fc
158/fc	1.00																						
65A/fc	0.64	1.00																					
11A/fc	0.69	0.75	1.00																				
33B/fc	0.65	0.76	0.73	1.00																			
64A/fc	0.68	0.66	0.76	0.61	1.00																		
118B/fc	0.62	0.67	0.74	0.61	0.81	1.00																	
17A/fc	0.63	0.68	0.76	0.62	0.80	0.82	1.00																
8G/fc	0.50	0.63	0.68	0.55	0.65	0.62	0.61	1.00															
7C/fc	0.58	0.60	0.65	0.53	0.69	0.62	0.64	0.83	1.00														
77A/ff	0.63	0.66	0.70	0.61	0.74	0.70	0.72	0.74	0.76	1.00													
34A/fc	0.59	0.65	0.68	0.57	0.72	0.65	0.65	0.80	0.77	0.78	1.00												
43A/d	0.49	0.60	0.65	0.57	0.65	0.63	0.60	0.72	0.70	0.74	0.78	1.00											
139B/fc	0.55	0.65	0.67	0.64	0.62	0.61	0.66	0.72	0.71	0.77	0.72	0.67	1.00										
58B/fc	0.67	0.68	0.68	0.62	0.66	0.58	0.61	0.76	0.78	0.74	0.74	0.70	0.77	1.00									
70/fc	0.50	0.67	0.66	0.66	0.60	0.57	0.60	0.73	0.70	0.70	0.69	0.67	0.83	0.79	1.00								
112A/fc	0.59	0.65	0.76	0.63	0.79	0.78	0.81	0.64	0.64	0.71	0.68	0.66	0.61	0.60	0.59	1.00							
148A/d	0.57	0.62	0.73	0.61	0.74	0.73	0.72	0.62	0.60	0.65	0.63	0.65	0.58	0.61	0.60	0.83	1.00						
9A/fc	0.61	0.71	0.77	0.69	0.79	0.74	0.75	0.69	0.65	0.70	0.70	0.66	0.70	0.70	0.70	0.75	0.75	1.00					
13A/fc	0.60	0.65	0.76	0.59	0.82	0.82	0.81	0.70	0.74	0.78	0.71	0.68	0.68	0.67	0.64	0.81	0.76	0.79	1.00				
15A/fc	0.56	0.65	0.71	0.58	0.78	0.73	0.75	0.74	0.74	0.76	0.81	0.78	0.67	0.70	0.67	0.75	0.68	0.76	0.83	1.00			
35A/fc	0.57	0.74	0.77	0.65	0.77	0.77	0.77	0.70	0.70	0.75	0.72	0.71	0.64	0.66	0.64	0.78	0.73	0.80	0.81	0.79	1.00		
105A/fc	0.64	0.62	0.63	0.68	0.56	0.60	0.64	0.54	0.55	0.63	0.52	0.50	0.67	0.60	0.68	0.58	0.58	0.66	0.61	0.56	0.60	1.00	
106A/fc	0.61	0.65	0.64	0.69	0.59	0.65	0.67	0.58	0.60	0.69	0.57	0.56	0.70	0.60	0.68	0.64	0.64	0.66	0.63	0.57	0.64	0.84	1.00

Similarity matrix from Data Set 7 (microcharacters only) with Gower's coefficient

	158/fc	65A/fc	11A/fc	33B/fc	64A/fc	118B/fc	17A/fc	8G/fc	7C/fc	77A/ff	34A/fc	43A/d	139B/fc	58B/fc	70/fc	112A/fc	148A/d	9A/fc	13A/fc	15A/fc	35A/fc	105A/fc	106A/fc
158/fc	1.00																						
65A/fc	0.63	1.00																					
11A/fc	0.62	0.81	1.00																				
33B/fc	0.51	0.83	0.77	1.00																			
64A/fc	0.70	0.67	0.72	0.58	1.00																		
118B/fc	0.71	0.71	0.73	0.55	0.84	1.00																	
17A/fc	0.64	0.65	0.71	0.64	0.67	0.62	1.00																
8G/fc	0.42	0.76	0.75	0.85	0.51	0.50	0.53	1.00															
7C/fc	0.53	0.71	0.76	0.74	0.57	0.59	0.53	0.79	1.00														
77A/ff	0.59	0.85	0.81	0.85	0.61	0.63	0.65	0.79	0.75	1.00													
34A/fc	0.59	0.85	0.79	0.81	0.62	0.64	0.59	0.78	0.75	0.89	1.00												
43A/d	0.55	0.82	0.80	0.80	0.62	0.64	0.61	0.79	0.76	0.84	0.87	1.00											
139B/fc	0.52	0.75	0.84	0.77	0.57	0.60	0.68	0.80	0.81	0.80	0.79	0.81	1.00										
58B/fc	0.57	0.80	0.86	0.80	0.63	0.63	0.68	0.80	0.79	0.81	0.79	0.83	0.82	1.00									
70/fc	0.41	0.72	0.76	0.79	0.50	0.51	0.58	0.87	0.76	0.80	0.78	0.83	0.85	0.80	1.00								
112A/fc	0.69	0.78	0.75	0.75	0.64	0.59	0.71	0.65	0.69	0.79	0.75	0.73	0.73	0.76	0.67	1.00							
148A/d	0.68	0.77	0.80	0.74	0.79	0.71	0.69	0.68	0.72	0.74	0.72	0.73	0.68	0.81	0.67	0.73	1.00						
9A/fc	0.63	0.73	0.74	0.72	0.69	0.63	0.63	0.60	0.65	0.72	0.74	0.67	0.66	0.73	0.61	0.70	0.80	1.00					
13A/fc	0.66	0.75	0.82	0.70	0.68	0.70	0.70	0.68	0.75	0.80	0.79	0.78	0.80	0.79	0.69	0.71	0.72	0.71	1.00				
15A/fc	0.58	0.81	0.78	0.78	0.64	0.65	0.70	0.71	0.67	0.81	0.85	0.84	0.83	0.77	0.76	0.76	0.70	0.70	0.81	1.00			
35A/fc	0.63	0.82	0.83	0.80	0.69	0.65	0.72	0.71	0.71	0.81	0.79	0.82	0.75	0.83	0.72	0.84	0.80	0.76	0.78	0.79	1.00		
105A/fc	0.52	0.62	0.70	0.67	0.53	0.53	0.61	0.73	0.71	0.72	0.67	0.67	0.74	0.74	0.69	0.62	0.68	0.60	0.70	0.64	0.69	1.00	
106A/fc	0.55	0.70	0.73	0.75	0.56	0.58	0.66	0.73	0.79	0.78	0.75	0.75	0.77	0.78	0.73	0.75	0.74	0.67	0.71	0.69	0.75	0.83	1.00

Similarity matrix from Data Set 8 (vegetative characters only) with Gower's coefficient

	158/fc	CHR	159A	65A/fc	66B/d	67A/fc	11A/fc	33B/fc	64A/fc	118B/f	17A/fc	8G/fc	7C/fc	5A/d	77A/ff	34A/fc	43A/d	139B/f	58B/fc	70/fc	112A/f	148A	9A/fc	13A/fc	13B/fc	4X/ff	15A/fc	35A/fc	134A	105Ad	105A/f	106A/f	108A/f
158/fc	1.00																															
CHR	0.81	1.00																														
159A/d	0.70	0.80	1.00																													
65A/fc	0.66	0.78	0.75	1.00																												
66B/d	0.66	0.70	0.69	0.75	1.00																											
67A/fc	0.75	0.68	0.67	0.76	0.81	1.00																										
11A/fc	0.74	0.67	0.70	0.71	0.78	0.84	1.00																									
33B/fc	0.73	0.79	0.77	0.84	0.79	0.75	0.68	1.00																								
64A/fc	0.68	0.63	0.62	0.61	0.74	0.80	0.81	0.60	1.00																							
118B/fc	0.61	0.61	0.59	0.61	0.78	0.72	0.76	0.59	0.86	1.00																						
17A/fc	0.65	0.60	0.62	0.63	0.73	0.77	0.81	0.58	0.90	0.88	1.00																					
8G/fc	0.58	0.58	0.57	0.60	0.69	0.63	0.74	0.56	0.69	0.75	0.73	1.00																				
7C/fc	0.63	0.55	0.55	0.54	0.59	0.62	0.67	0.52	0.72	0.68	0.72	0.87	1.00																			
5A/d	0.54	0.56	0.53	0.53	0.65	0.59	0.65	0.50	0.69	0.71	0.68	0.85	0.84	1.00																		
77A/ff	0.67	0.61	0.60	0.57	0.62	0.68	0.75	0.54	0.77	0.76	0.76	0.80	0.84	0.81	1.00																	
34A/fc	0.62	0.61	0.58	0.62	0.69	0.68	0.72	0.58	0.79	0.76	0.78	0.78	0.79	0.79	0.81	1.00																
43A/d	0.51	0.52	0.46	0.48	0.65	0.54	0.63	0.48	0.65	0.67	0.61	0.72	0.71	0.81	0.75	0.74	1.00															
139B/fc	0.59	0.68	0.70	0.65	0.58	0.58	0.64	0.64	0.56	0.57	0.59	0.70	0.68	0.63	0.72	0.64	0.54	1.00														
58B/fc	0.72	0.69	0.70	0.66	0.68	0.66	0.70	0.69	0.62	0.58	0.61	0.68	0.69	0.61	0.74	0.67	0.63	0.79	1.00													
70/fc	0.56	0.69	0.75	0.67	0.60	0.57	0.57	0.67	0.52	0.49	0.52	0.66	0.66	0.59	0.62	0.58	0.54	0.84	0.80	1.00												
112A/fc	0.58	0.57	0.56	0.52	0.69	0.70	0.76	0.52	0.87	0.86	0.87	0.69	0.68	0.68	0.74	0.73	0.68	0.50	0.54	0.45	1.00											
148A/d	0.58	0.59	0.66	0.54	0.72	0.67	0.73	0.53	0.75	0.76	0.73	0.66	0.61	0.69	0.67	0.66	0.65	0.49	0.56	0.49	0.82	1.00										
9A/fc	0.64	0.75	0.73	0.71	0.72	0.72	0.79	0.70	0.82	0.81	0.80	0.71	0.64	0.64	0.67	0.69	0.57	0.68	0.65	0.63	0.74	0.69	1.00									
13A/fc	0.60	0.56	0.61	0.57	0.65	0.70	0.76	0.50	0.85	0.84	0.84	0.74	0.77	0.75	0.82	0.73	0.66	0.60	0.61	0.52	0.82	0.77	0.78	1.00								
13B/fc	0.61	0.63	0.61	0.58	0.68	0.73	0.78	0.57	0.85	0.84	0.81	0.75	0.73	0.76	0.77	0.75	0.67	0.57	0.59	0.53	0.82	0.79	0.87	0.86	1.00							
4X/ff	0.52	0.53	0.59	0.51	0.68	0.63	0.72	0.49	0.80	0.80	0.76	0.74	0.73	0.76	0.74	0.76	0.73	0.53	0.58	0.53	0.81	0.80	0.73	0.82	0.83	1.00						
15A/fc	0.57	0.53	0.56	0.63	0.69	0.71	0.73	0.55	0.85	0.82	0.84	0.76	0.79	0.78	0.81	0.84	0.72	0.59	0.64	0.56	0.80	0.69	0.74	0.88	0.84	0.80	1.00					
35A/fc	0.57	0.56	0.55	0.65	0.72	0.68	0.70	0.56	0.83	0.86	0.81	0.76	0.75	0.77	0.78	0.78	0.71	0.55	0.59	0.51	0.80	0.74	0.78	0.88	0.85	0.79	0.88	1.00				
134A/d	0.55	0.61	0.63	0.52	0.70	0.63	0.75	0.49	0.78	0.80	0.75	0.67	0.61	0.74	0.70	0.68	0.69	0.53	0.55	0.49	0.81	0.85	0.73	0.79	0.84	0.80	0.75	0.75	1.00			
105A/d	0.60	0.68	0.74	0.56	0.54	0.53	0.58	0.57	0.55	0.57	0.58	0.64	0.68	0.66	0.66	0.62	0.52	0.70	0.62	0.67	0.52	0.61	0.62	0.62	0.60	0.59	0.58	0.53	0.60	1.00		
105A/fc	0.72	0.84	0.79	0.72	0.68	0.69	0.69	0.74	0.65	0.62	0.64	0.66	0.66	0.61	0.68	0.64	0.53	0.78	0.76	0.81	0.58	0.57	0.79	0.60	0.68	0.59	0.63	0.57	0.61	0.79	1.00	
106A/fc	0.67	0.80	0.80	0.70	0.66	0.65	0.67	0.74	0.65	0.65	0.65	0.71	0.68	0.68	0.71	0.65	0.58	0.73	0.69	0.75	0.62	0.64	0.74	0.65	0.71	0.65	0.65	0.63	0.65	0.81	0.86	1.00
108A/fc	0.68	0.62	0.63	0.58	0.69	0.73	0.84	0.59	0.78	0.77	0.76	0.77	0.74	0.76	0.82	0.73	0.70	0.60	0.66	0.56	0.79	0.75	0.75	0.80	0.83	0.76	0.79	0.74	0.79	0.63	0.72	0.72

Similarity matrix from Data Set 9 (floral characters only) with Gower's coefficient

	CHR	65A/fc	66B/d	67A/fc	11A/fc	33B/fc	64A/fc	118B/f	17A/fc	8G/fc	7C/fc	5A/d	77A/ff	34A/fc	43A/d	139B/f	58B/fc	70/fc	112A/f	148A/d	9A/fc	13A/fc	13B/fc	4X/ff	15A/fc	35A/fc	134A/d	105A/d	105A/f	106A/f	108A/f
CHR	1.00																														
65A/fc	0.71	1.00																													
66B/d	0.74	0.66	1.00																												
67A/fc	0.75	0.78	0.76	1.00																											
11A/fc	0.72	0.79	0.74	0.78	1.00																										
33B/fc	0.66	0.73	0.69	0.79	0.79	1.00																									
64A/fc	0.74	0.71	0.78	0.75	0.74	0.64	1.00																								
118B/fc	0.74	0.74	0.70	0.82	0.74	0.65	0.80	1.00																							
17A/fc	0.77	0.73	0.73	0.82	0.73	0.66	0.74	0.79	1.00																						
8G/fc	0.68	0.66	0.63	0.62	0.66	0.58	0.65	0.56	0.57	1.00																					
7C/fc	0.71	0.66	0.62	0.64	0.65	0.59	0.69	0.62	0.61	0.82	1.00																				
5A/d	0.71	0.63	0.67	0.66	0.65	0.54	0.71	0.66	0.65	0.82	0.81	1.00																			
77A/ff	0.74	0.73	0.76	0.78	0.68	0.69	0.74	0.69	0.72	0.72	0.73	0.69	1.00																		
34A/fc	0.63	0.70	0.69	0.68	0.67	0.61	0.70	0.62	0.59	0.82	0.77	0.81	0.78	1.00																	
43A/d	0.65	0.70	0.70	0.73	0.71	0.67	0.69	0.64	0.63	0.75	0.73	0.79	0.77	0.83	1.00																
139B/fc	0.68	0.67	0.77	0.76	0.71	0.68	0.69	0.67	0.73	0.74	0.76	0.73	0.82	0.79	0.78	1.00															
58B/fc	0.67	0.70	0.65	0.64	0.70	0.60	0.71	0.62	0.64	0.84	0.84	0.83	0.75	0.80	0.78	0.77	1.00														
70/fc	0.70	0.70	0.72	0.75	0.74	0.69	0.68	0.65	0.69	0.80	0.75	0.77	0.76	0.78	0.79	0.85	0.79	1.00													
112A/fc	0.78	0.76	0.78	0.80	0.78	0.72	0.75	0.73	0.79	0.63	0.64	0.69	0.71	0.67	0.68	0.71	0.68	0.72	1.00												
148A/d	0.76	0.72	0.76	0.75	0.76	0.70	0.77	0.73	0.75	0.63	0.64	0.69	0.68	0.66	0.67	0.68	0.69	0.71	0.85	1.00											
9A/fc	0.77	0.74	0.76	0.78	0.77	0.70	0.79	0.71	0.73	0.70	0.69	0.73	0.74	0.74	0.75	0.74	0.76	0.77	0.77	0.81	1.00										
13A/fc	0.83	0.74	0.76	0.83	0.78	0.68	0.81	0.81	0.80	0.69	0.74	0.75	0.78	0.74	0.73	0.76	0.74	0.75	0.80	0.77	0.80	1.00									
13B/fc	0.79	0.73	0.73	0.77	0.77	0.63	0.70	0.73	0.76	0.71	0.66	0.68	0.70	0.68	0.68	0.70	0.68	0.75	0.74	0.69	0.79	0.79	1.00								
4X/ff	0.73	0.70	0.73	0.75	0.73	0.63	0.79	0.72	0.72	0.79	0.77	0.82	0.74	0.81	0.85	0.75	0.80	0.81	0.76	0.73	0.83	0.81	0.74	1.00							
15A/fc	0.77	0.69	0.70	0.70	0.71	0.64	0.75	0.68	0.69	0.75	0.74	0.83	0.75	0.81	0.86	0.75	0.78	0.77	0.73	0.71	0.78	0.80	0.74	0.89	1.00						
35A/fc	0.75	0.82	0.76	0.81	0.83	0.72	0.75	0.73	0.77	0.69	0.68	0.68	0.76	0.71	0.74	0.72	0.74	0.75	0.78	0.75	0.82	0.78	0.85	0.76	0.76	1.00					
134A/d	0.68	0.65	0.73	0.71	0.74	0.70	0.72	0.76	0.73	0.60	0.59	0.69	0.62	0.64	0.69	0.68	0.62	0.76	0.79	0.81	0.76	0.74	0.67	0.74	0.73	0.72	1.00				
105A/d	0.77	0.68	0.76	0.79	0.82	0.80	0.65	0.71	0.74	0.56	0.52	0.50	0.75	0.54	0.62	0.65	0.50	0.64	0.74	0.69	0.76	0.74	0.85	0.73	0.68	0.81	0.60	1.00			
105A/fc	0.62	0.58	0.62	0.69	0.62	0.66	0.53	0.62	0.67	0.50	0.53	0.46	0.63	0.48	0.52	0.63	0.53	0.62	0.61	0.61	0.60	0.64	0.64	0.55	0.55	0.65	0.60	0.84	1.00		
106A/fc	0.58	0.63	0.67	0.68	0.64	0.69	0.58	0.67	0.71	0.51	0.57	0.45	0.71	0.53	0.57	0.70	0.57	0.66	0.67	0.66	0.61	0.64	0.58	0.51	0.55	0.67	0.66	0.76	0.84	1.00	
108A/fc	0.65	0.61	0.63	0.72	0.62	0.65	0.56	0.71	0.73	0.46	0.50	0.50	0.64	0.46	0.50	0.63	0.49	0.64	0.64	0.62	0.61	0.66	0.66	0.58	0.54	0.67	0.63	0.78	0.87	0.88	1.00

Similarity matrix from the molecular Data Set with Jaccard's coefficient

	8G	7C	34A	43A	70B	58B	139B	9A	13A	11A	112A	148A	15A	64A	35A	118B	17A	65A	159	33B	105A	106A	158A	*Ca.*	*Oz.*	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
8G	1.00																																				
7C	0.86	1.00																																			
34A	0.76	0.78	1.00																																		
43A	0.78	0.79	0.87	1.00																																	
70B	0.72	0.75	0.75	0.71	1.00																																
58B	0.74	0.73	0.69	0.70	0.81	1.00																															
139B	0.69	0.71	0.71	0.69	0.84	0.80	1.00																														
9A	0.58	0.60	0.58	0.57	0.63	0.62	0.61	1.00																													
13A	0.64	0.67	0.64	0.63	0.67	0.63	0.67	0.76	1.00																												
11A	0.62	0.61	0.58	0.60	0.60	0.60	0.58	0.78	0.74	1.00																											
112A	0.63	0.62	0.60	0.65	0.61	0.62	0.61	0.65	0.70	0.74	1.00																										
148A	0.63	0.61	0.60	0.62	0.62	0.63	0.64	0.63	0.68	0.75	0.80	1.00																									
15A	0.61	0.64	0.65	0.64	0.63	0.59	0.64	0.61	0.68	0.65	0.67	0.69	1.00																								
64A	0.59	0.59	0.60	0.61	0.61	0.61	0.62	0.69	0.74	0.74	0.69	0.71	0.76	1.00																							
35A	0.61	0.61	0.59	0.61	0.60	0.59	0.60	0.70	0.74	0.81	0.73	0.71	0.68	0.76	1.00																						
118B	0.61	0.62	0.63	0.62	0.61	0.58	0.61	0.65	0.73	0.69	0.68	0.66	0.72	0.72	0.73	1.00																					
17A	0.60	0.63	0.62	0.61	0.61	0.58	0.62	0.66	0.69	0.70	0.68	0.69	0.71	0.70	0.75	0.79	1.00																				
65A	0.62	0.60	0.61	0.63	0.60	0.58	0.59	0.71	0.68	0.77	0.69	0.68	0.68	0.73	0.75	0.71	0.72	1.00																			
159	0.63	0.59	0.61	0.63	0.59	0.56	0.58	0.60	0.61	0.64	0.68	0.64	0.66	0.63	0.67	0.64	0.68	0.80	1.00																		
33B	0.58	0.59	0.61	0.61	0.58	0.54	0.58	0.63	0.66	0.64	0.64	0.62	0.66	0.63	0.65	0.62	0.65	0.72	0.70	1.00																	
105A	0.62	0.60	0.63	0.64	0.72	0.65	0.66	0.58	0.62	0.57	0.61	0.61	0.63	0.61	0.62	0.62	0.61	0.65	0.63	0.60	1.00																
106A	0.61	0.60	0.62	0.63	0.70	0.64	0.64	0.57	0.63	0.59	0.62	0.63	0.64	0.63	0.61	0.61	0.61	0.64	0.62	0.61	0.92	1.00															
158A	0.52	0.50	0.53	0.54	0.50	0.48	0.50	0.51	0.53	0.57	0.57	0.56	0.53	0.54	0.59	0.56	0.56	0.57	0.57	0.58	0.55	0.53	1.00														
Ca.	0.27	0.26	0.26	0.27	0.26	0.26	0.25	0.25	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.28	0.28	0.27	0.27	0.25	1.00													
Oz.	0.42	0.41	0.41	0.42	0.41	0.40	0.41	0.40	0.42	0.42	0.43	0.43	0.44	0.42	0.43	0.44	0.46	0.46	0.46	0.45	0.42	0.40	0.43	0.27	1.00												
P1	0.62	0.65	0.60	0.58	0.61	0.59	0.60	0.57	0.62	0.59	0.60	0.65	0.67	0.61	0.60	0.63	0.64	0.56	0.54	0.57	0.55	0.57	0.46	0.23	0.39	1.00											
P2	0.64	0.68	0.62	0.61	0.63	0.61	0.63	0.59	0.65	0.61	0.61	0.65	0.68	0.61	0.61	0.63	0.65	0.56	0.54	0.58	0.57	0.59	0.46	0.24	0.39	0.93	1.00										
P3	0.65	0.64	0.60	0.61	0.60	0.61	0.60	0.56	0.62	0.60	0.61	0.64	0.65	0.62	0.60	0.60	0.61	0.57	0.55	0.56	0.56	0.59	0.46	0.24	0.39	0.91	0.93	1.00									
P4	0.63	0.65	0.61	0.59	0.62	0.60	0.61	0.59	0.65	0.61	0.62	0.65	0.68	0.63	0.61	0.61	0.64	0.58	0.55	0.60	0.55	0.59	0.46	0.24	0.39	0.88	0.92	0.91	1.00								
P5	0.63	0.67	0.62	0.60	0.62	0.61	0.62	0.58	0.64	0.60	0.62	0.64	0.69	0.63	0.61	0.64	0.65	0.56	0.55	0.58	0.54	0.57	0.46	0.24	0.38	0.91	0.93	0.90	0.92	1.00							
P6	0.59	0.62	0.58	0.56	0.58	0.57	0.59	0.58	0.63	0.59	0.59	0.62	0.66	0.61	0.61	0.62	0.63	0.59	0.55	0.56	0.52	0.54	0.44	0.23	0.38	0.83	0.85	0.82	0.88	0.86	1.00						
P7	0.61	0.64	0.58	0.57	0.58	0.57	0.58	0.58	0.64	0.60	0.62	0.64	0.67	0.60	0.61	0.62	0.65	0.56	0.55	0.59	0.53	0.55	0.45	0.24	0.39	0.86	0.89	0.88	0.86	0.89	0.89	1.00					
P8	0.65	0.65	0.60	0.60	0.60	0.59	0.60	0.59	0.64	0.61	0.63	0.63	0.67	0.61	0.60	0.64	0.65	0.58	0.56	0.59	0.53	0.56	0.44	0.25	0.38	0.87	0.89	0.87	0.89	0.90	0.84	0.88	1.00				
P9	0.64	0.67	0.59	0.59	0.60	0.60	0.60	0.58	0.63	0.59	0.61	0.62	0.67	0.60	0.58	0.62	0.64	0.56	0.53	0.57	0.53	0.55	0.44	0.25	0.38	0.89	0.90	0.87	0.88	0.88	0.86	0.87	0.94	1.00			
P10	0.65	0.63	0.59	0.60	0.60	0.62	0.60	0.57	0.62	0.62	0.63	0.66	0.65	0.63	0.60	0.60	0.62	0.57	0.54	0.56	0.55	0.58	0.46	0.25	0.38	0.87	0.90	0.92	0.89	0.89	0.85	0.86	0.94	0.94	1.00		
P11	0.65	0.67	0.61	0.61	0.61	0.60	0.61	0.59	0.65	0.60	0.64	0.63	0.66	0.59	0.59	0.64	0.64	0.56	0.54	0.58	0.54	0.56	0.46	0.25	0.38	0.86	0.90	0.87	0.89	0.90	0.86	0.87	0.93	0.92	0.89	1.00	
P12	0.64	0.64	0.59	0.60	0.60	0.62	0.61	0.58	0.62	0.62	0.63	0.67	0.65	0.62	0.60	0.60	0.62	0.59	0.56	0.57	0.55	0.58	0.46	0.24	0.38	0.86	0.88	0.88	0.89	0.88	0.87	0.86	0.88	0.89	0.92	0.89	1.00

Similarity matrix from a morphological data set including only the 23 OTUs included in the molecular study

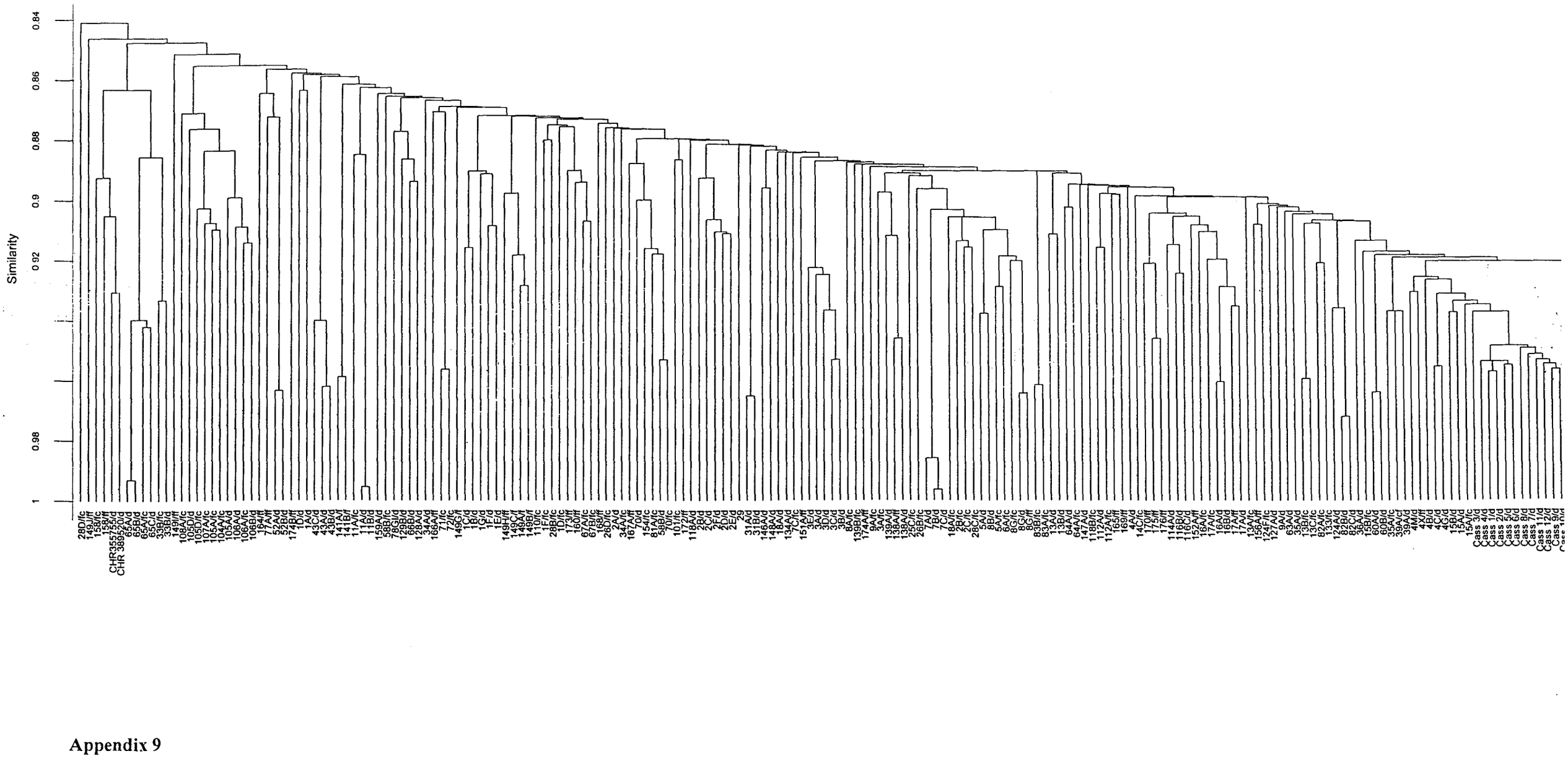
	8G/fc	7C/fc	34A/fc	43A/d	70/fc	58B/fc	139B/fc	9A/fc	13A/fc	11A/fc	112A/fc	148A/d	15A/fc	64A/fc	35A/fc	118B/fc	17A/fc	65A/fc	159A/d	33B/fc	105A/fc	106A/fc	158/fc
8G/fc	1.00																						
7C/fc	0.83	1.00																					
34A/fc	0.79	0.76	1.00																				
43A/d	0.71	0.68	0.77	1.00																			
70/fc	0.72	0.68	0.68	0.65	1.00																		
58B/fc	0.76	0.78	0.72	0.68	0.79	1.00																	
139B/fc	0.71	0.69	0.70	0.65	0.83	0.76	1.00																
9A/fc	0.72	0.65	0.69	0.66	0.72	0.70	0.72	1.00															
13A/fc	0.70	0.73	0.70	0.67	0.63	0.66	0.66	0.81	1.00														
11A/fc	0.67	0.62	0.65	0.63	0.64	0.65	0.65	0.78	0.74	1.00													
112A/fc	0.64	0.63	0.66	0.65	0.58	0.58	0.59	0.76	0.82	0.76	1.00												
148A/d	0.63	0.58	0.62	0.64	0.60	0.58	0.58	0.75	0.77	0.73	0.84	1.00											
15A/fc	0.75	0.74	0.80	0.78	0.66	0.69	0.64	0.77	0.82	0.69	0.74	0.69	1.00										
64A/fc	0.67	0.71	0.73	0.66	0.62	0.67	0.62	0.81	0.85	0.76	0.82	0.73	0.80	1.00									
35A/fc	0.70	0.68	0.70	0.69	0.62	0.63	0.61	0.80	0.82	0.75	0.77	0.72	0.80	0.79	1.00								
118B/fc	0.64	0.61	0.65	0.64	0.58	0.56	0.61	0.76	0.83	0.74	0.81	0.73	0.73	0.80	0.79	1.00							
17A/fc	0.64	0.65	0.66	0.60	0.62	0.60	0.67	0.77	0.82	0.77	0.82	0.74	0.75	0.82	0.78	0.85	1.00						
65A/fc	0.59	0.56	0.61	0.55	0.65	0.64	0.62	0.70	0.63	0.73	0.62	0.59	0.61	0.66	0.72	0.67	0.68	1.00					
159A/d	0.54	0.51	0.56	0.42	0.73	0.67	0.68	0.70	0.57	0.69	0.52	0.63	0.55	0.58	0.51	0.57	0.60	0.74	1.00				
33B/fc	0.51	0.49	0.53	0.53	0.65	0.58	0.63	0.68	0.57	0.74	0.60	0.60	0.55	0.61	0.62	0.63	0.63	0.74	0.76	1.00			
105A/fc	0.51	0.53	0.48	0.46	0.67	0.58	0.65	0.66	0.59	0.61	0.58	0.56	0.54	0.57	0.59	0.61	0.65	0.61	0.76	0.67	1.00		
106A/fc	0.56	0.55	0.52	0.51	0.67	0.57	0.68	0.66	0.61	0.62	0.63	0.61	0.55	0.60	0.62	0.67	0.68	0.64	0.79	0.69	0.84	1.00	
158/fc	0.54	0.61	0.59	0.47	0.54	0.72	0.57	0.61	0.57	0.72	0.55	0.53	0.55	0.67	0.55	0.58	0.63	0.65	0.68	0.72	0.70	0.64	1.00

Similarity matrix from morphological data including only the 12 representatives of the Cass population (P1-P12) with Gower's coefficient

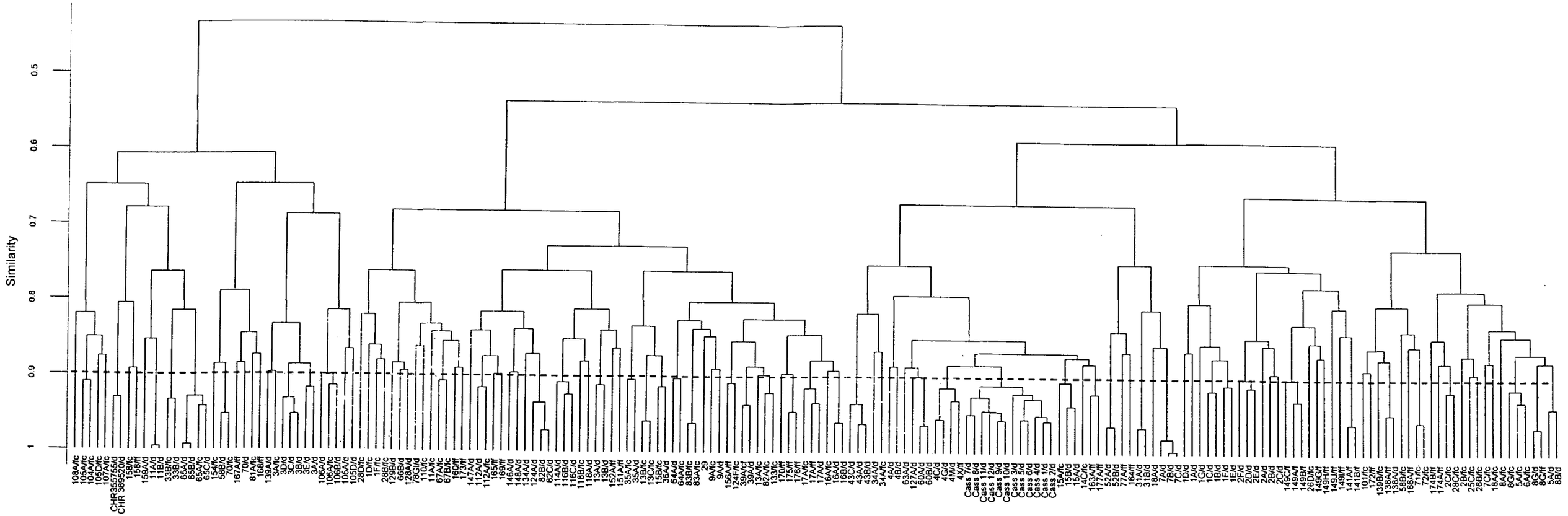
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	1.00											
P2	0.96	1.00										
P3	0.94	0.93	1.00									
P4	0.95	0.95	0.94	1.00								
P5	0.95	0.95	0.93	0.95	1.00							
P6	0.93	0.94	0.92	0.94	0.95	1.00						
P7	0.93	0.94	0.93	0.94	0.95	0.92	1.00					
P8	0.92	0.93	0.92	0.93	0.93	0.91	0.95	1.00				
P9	0.92	0.94	0.93	0.94	0.94	0.93	0.95	0.94	1.00			
P10	0.92	0.93	0.93	0.92	0.94	0.92	0.94	0.95	0.96	1.00		
P11	0.91	0.93	0.93	0.91	0.94	0.93	0.93	0.93	0.94	0.95	1.00	
P12	0.91	0.92	0.92	0.92	0.94	0.92	0.93	0.93	0.94	0.95	0.94	1.00

Similarity matrix from AFLP data including only the 12 representatives of the Cass population (P1-P12) with Jaccard's coefficient

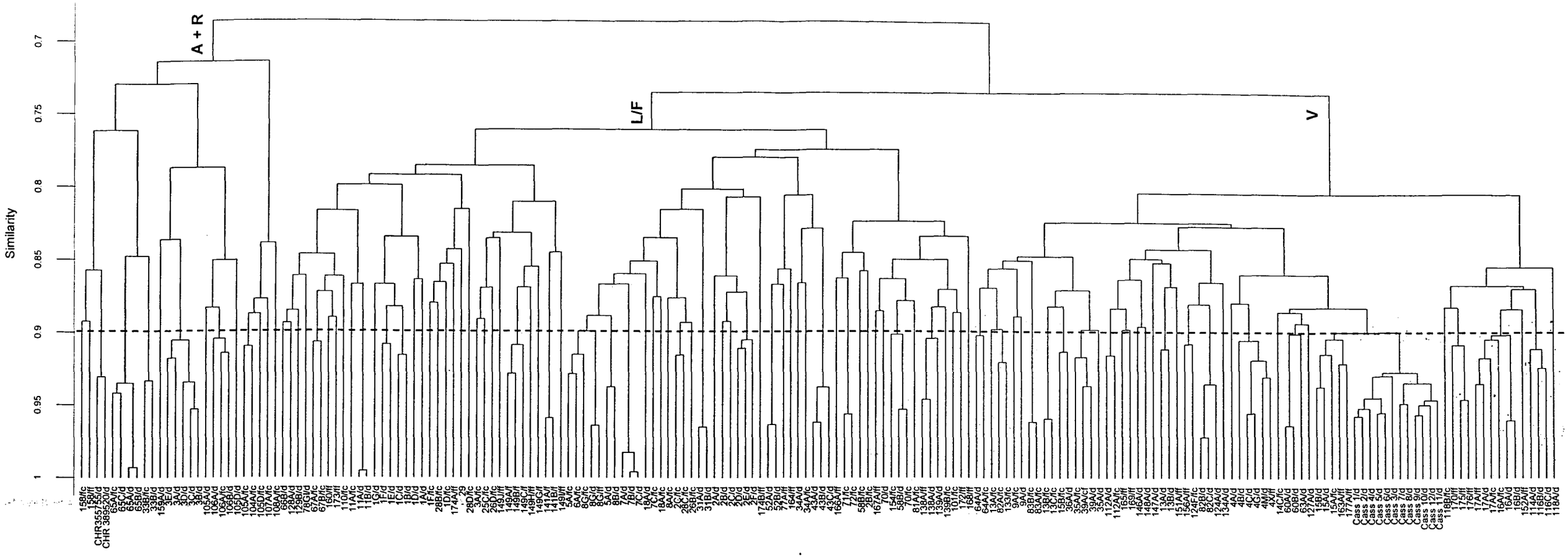
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12.
P1	1.00											
P2	0.93	1.00										
P3	0.91	0.93	1.00									
P4	0.88	0.92	0.91	1.00								
P5	0.91	0.93	0.90	0.92	1.00							
P6	0.83	0.85	0.82	0.88	0.86	1.00						
P7	0.86	0.89	0.88	0.86	0.89	0.84	1.00					
P8	0.87	0.89	0.87	0.89	0.90	0.84	0.88	1.00				
P9	0.89	0.90	0.87	0.88	0.88	0.86	0.87	0.94	1.00			
P10	0.87	0.90	0.92	0.89	0.89	0.85	0.86	0.90	0.94	1.00		
P11	0.86	0.90	0.87	0.89	0.90	0.86	0.87	0.93	0.92	0.89	1.00	
P12	0.86	0.88	0.88	0.89	0.88	0.87	0.86	0.88	0.89	0.92	0.89	1.00


Similarity
1
0.98
0.92
0.9
0.88
0.86
0.84
28D/fc
149J/ff
158/fc
CHR355755/d
CHR 389520/d
65A/d
65B/d
65A/fc
65C/d
33B/fc
33B/d
148A/ff
104A/fc
105D/d
105D/fc
107A/fc
105A/fc
104A/fc
105A/d
106A/d
106A/fc
106B/d
164/ff
77A/ff
52A/d
52B/d
174B/ff
1D/d
1A/d
43C/d
43A/d
43B/d
141B/f
11A/fc
11A/d
11B/d
159A/d
58B/fc
78G/d
129B/d
66B/d
128A/d
168A/d
71/fc
72/fc
149G/f
1C/d
1B/d
1G/d
1F/d
1E/d
149H/ff
149C/f
149B/f
110/fc
1F/fc
28B/fc
1D/fc
173/ff
160/ff
67A/fc
67B/fc
26D/fc
2A/d
34A/fc
167A/ff
70/d
154/fc
81A/fc
58B/d
101/fc
172/ff
118A/d
2B/d
2C/d
2F/d
2D/d
2E/d
29
31A/d
31B/d
146A/d
148A/d
18A/d
134A/d
7C/fc
151A/ff
3E/d
3A/d
3C/d
3B/d
8A/fc
139B/fc
174A/ff
9A/fc
3A/fc
139A/d
138A/ff
138C/d
26B/fc
7A/d
7B/d
7C/d
18A/fc
2B/fc
2C/fc
28C/fc
5A/d
6B/d
6A/fc
8G/fc
8G/d
8G/ff
83B/fc
83A/fc
13A/d
13B/d
64A/d
64A/fc
147A/d
118B/fc
112A/d
112A/fc
165/ff
169/ff
4A/d
14C/fc
170/ff
175/ff
114A/d
116B/d
116C/d
152A/ff
16A/fc
17A/fc
16A/d
16B/d
17A/ff
17A/d
134A/d
156A/ff
124F/fc
127A/d
9A/d
63A/d
35A/d
13B/fc
13C/fc
82A/fc
123A/fc
123A/d
82B/d
82C/d
36A/d
15B/fc
60A/d
60B/d
35A/fc
39A/d
39A/d
44A/d
4X/ff
4B/d
4C/d
4G/d
15B/d
15A/d
15A/fc
Cass 3/d
Cass 1/d
Cass 1/d
Cass 2/d
Cass 5/d
Cass 6/d
Cass 8/d
Cass 7/d
Cass 11/d
Cass 12/d
Cass 9/d

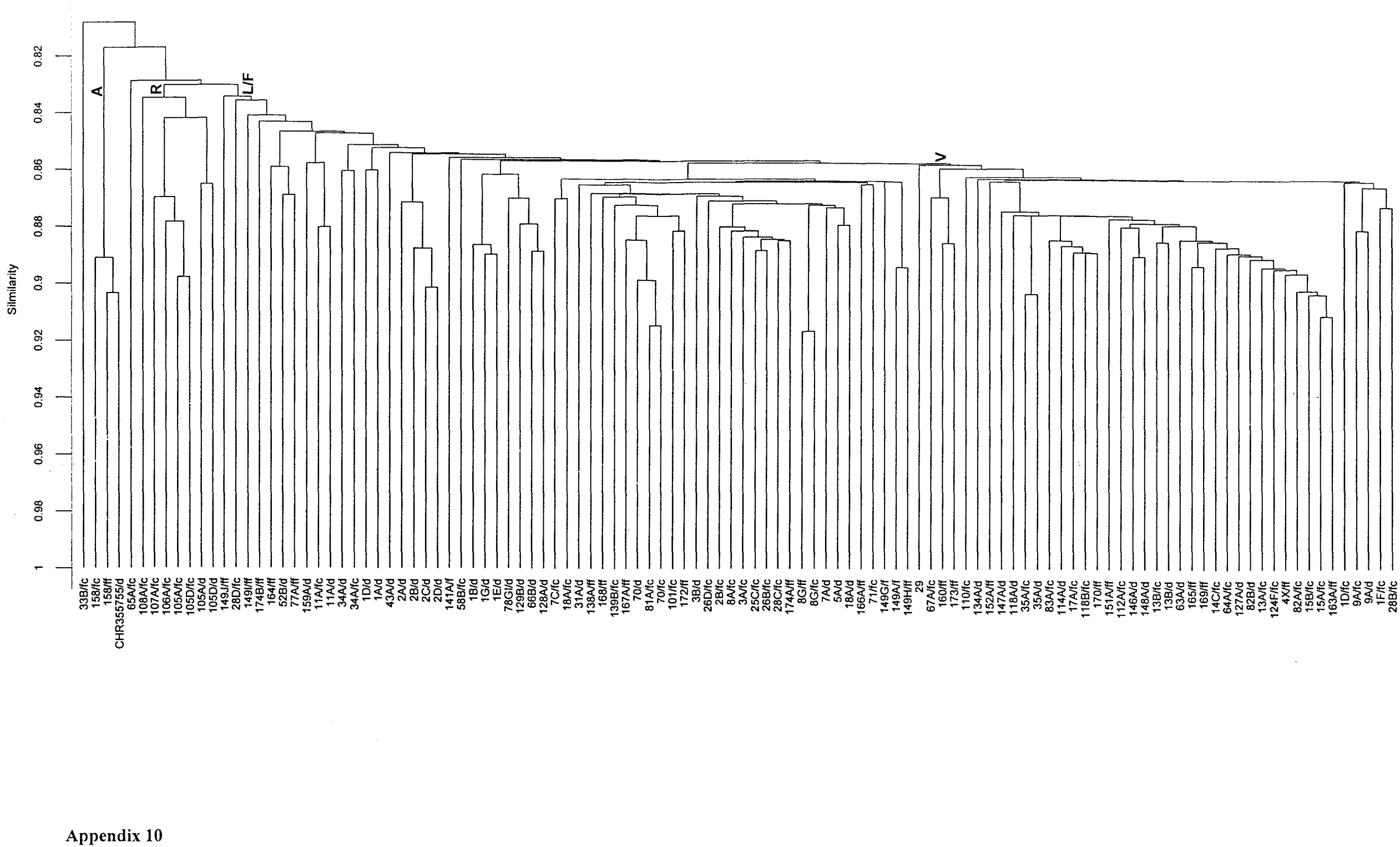
Appendix 9


Similarity
1
0.9
0.8
0.7
0.6
0.5
108A/fc
105A/fc
104A/fc
107A/fc
107A/fc
CHR355755/d
CHR 389520/d
158/fc
158/ff
159A/d
11A/d
11B/d
33B/fc
33B/d
65A/d
65B/d
65A/fc
65C/d
154/fc
58B/d
70/fc
167A/ff
70/d
81A/fc
168/ff
139A/d
3A/fc
3D/d
3C/d
3E/d
3E/d
3A/d
106A/d
106A/fc
106B/d
105A/d
105D/d
28D/fc
1D/fc
1F/fc
29B/fc
129B/d
66B/d
128A/d
78G/d
110/fc
11A/fc
67A/fc
67B/fc
160/ff
17A/ff
147A/d
112A/d
112A/fc
165/ff
169/ff
146A/d
148A/d
134A/d
124A/d
82B/d
82A/d
114A/d
116B/d
116C/d
116B/fc
118A/d
13A/d
13B/d
152A/ff
151A/ff
35A/fc
35A/d
13B/fc
13C/fc
15B/fc
36A/d
64A/d
64A/fc
83B/fc
83A/fc
29
9A/fc
9A/d
156A/ff
124F/fc
39A/d
39A/fc
82A/fc
133/ff
170/ff
175/ff
176/ff
17A/fc
17A/ff
17A/d
16A/fc
16A/d
16B/d
43C/d
43A/d
43B/d
34A/d
34A/fc
4A/d
4B/d
63A/d
127A/d
60A/d
60B/d
4C/d
4G/d
4M/d
4X/ff
Cass 7/d
Cass 8/d
Cass 11/d
Cass 12/d
Cass 9/d
Cass 10/d
Cass 3/d
Cass 5/d
Cass 6/d
Cass 4/d
Cass 1/d
Cass 2/d
15A/fc
15B/d
15A/d
14C/fc
163A/ff
177A/ff
52A/d
52B/d
77A/ff
16A/ff
31B/d
18A/d
7A/d
7B/d
7C/d
1D/d
1A/d
1G/d
1C/d
1B/d
1E/d
2F/d
2D/d
2E/d
2A/d
2B/d
2C/d
149C/ff
149A/ff
149B/ff
26D/fc
149G/ff
149H/ff
149J/ff
149I/ff
141A/ff
141B/ff
172/ff
139B/fc
138A/ff
138A/d
58B/fc
166A/ff
71/fc
71/fc
174B/ff
174A/ff
2C/fc
28C/fc
2B/fc
25C/fc
26B/fc
7C/fc
18A/fc
8A/fc
8G/fc
5A/fc
6A/fc
8G/d
8G/ff
5A/d
8B/d

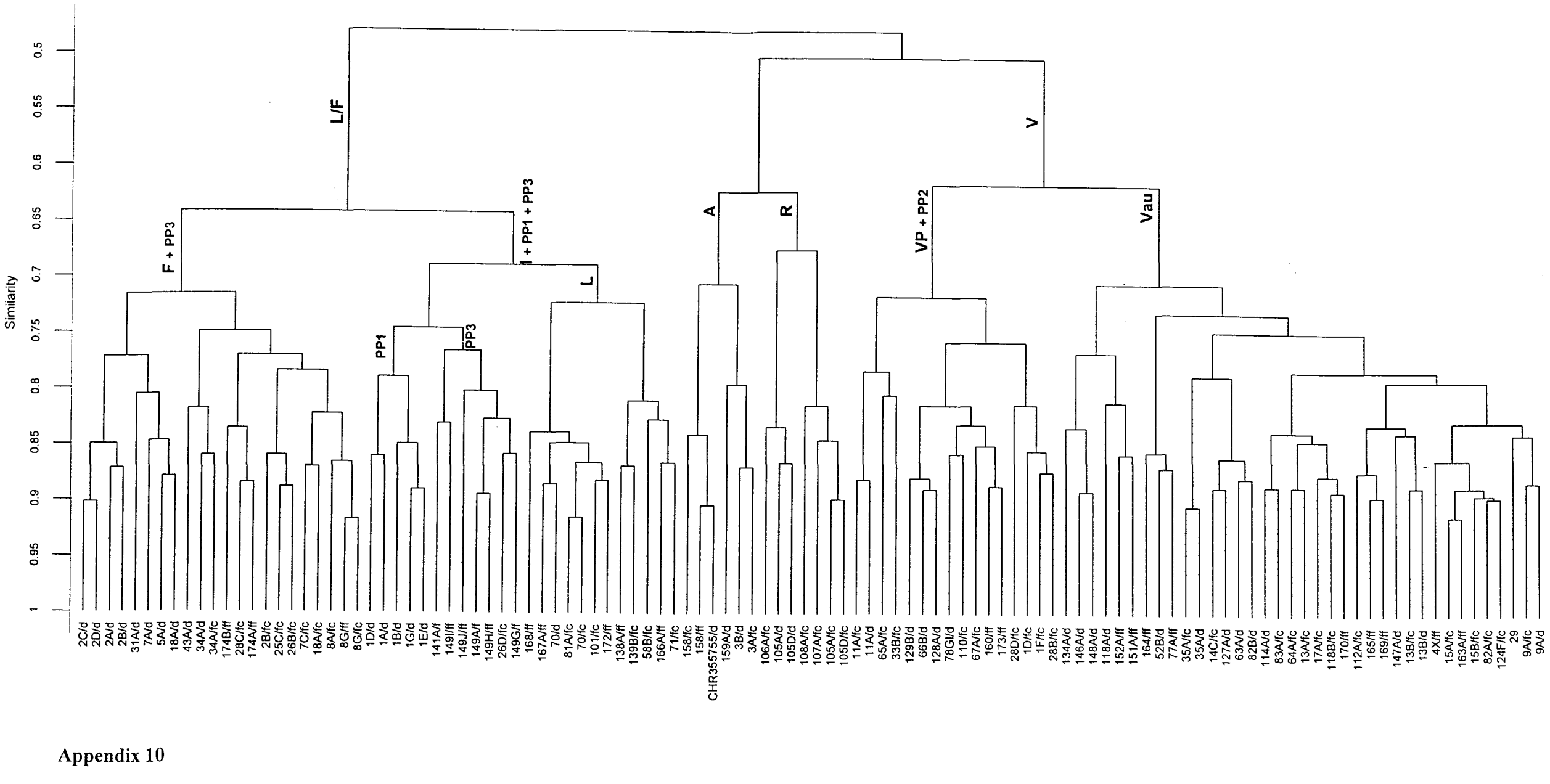
Appendix 9



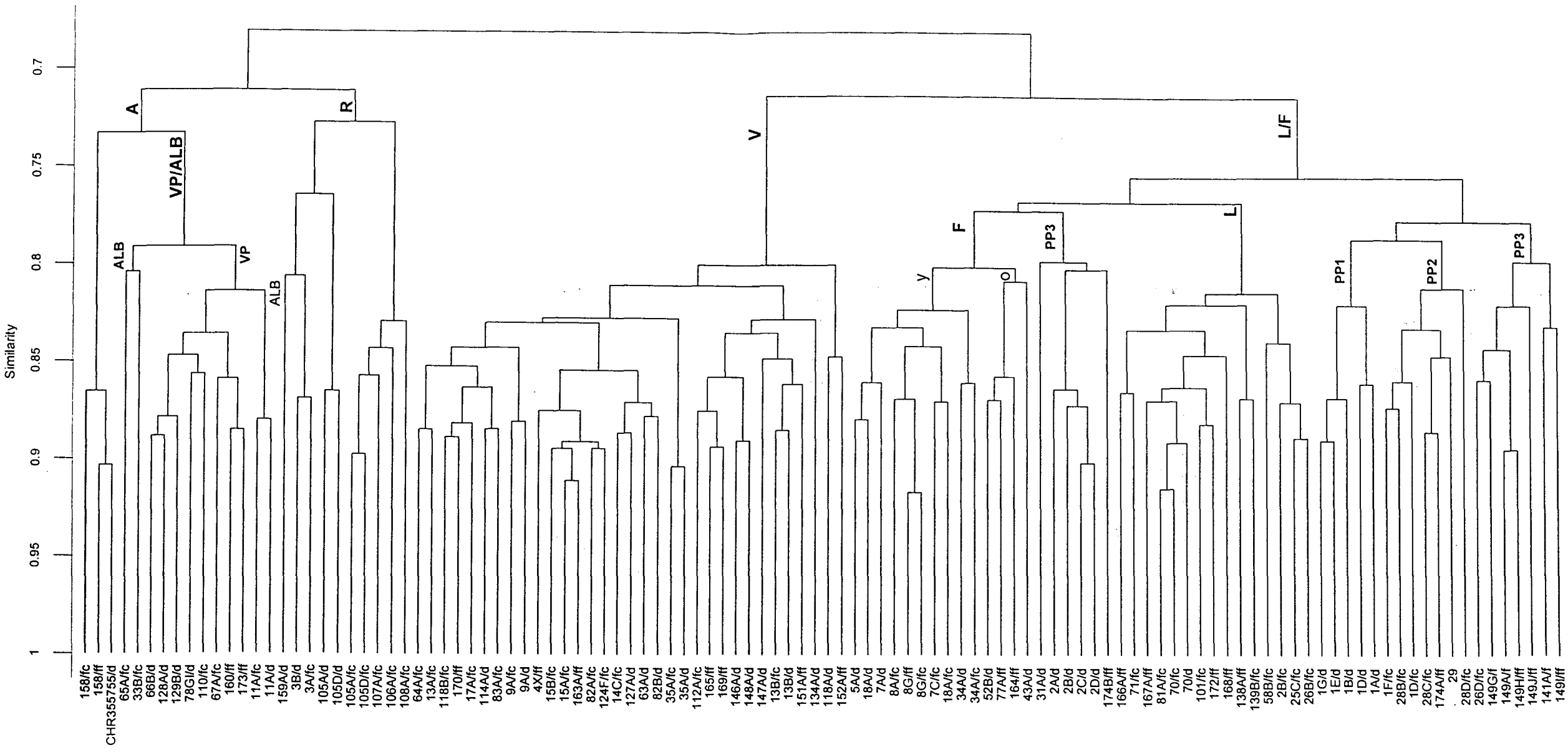
Appendix 9


Silmilarity
1
0.98
0.96
0.94
0.92
0.9
0.88
0.86
0.84
0.82
A
R
L/F
V
33B/fc
158/fc
158/ff
CHR355755/d
65A/fc
108A/fc
107A/fc
106A/fc
105A/fc
105D/fc
105A/d
105D/d
149J/ff
28D/fc
149I/ff
174B/ff
164/ff
52B/d
77A/ff
159A/d
11A/fc
11A/d
34A/d
34A/fc
1D/d
1A/d
43A/d
2A/d
2B/d
2C/d
2D/d
141A/f
58B/fc
1B/d
1G/d
1E/d
78G/d
129B/d
66B/d
128A/d
7C/fc
18A/fc
31A/d
138A/ff
168/ff
139B/fc
167A/ff
70/d
81A/fc
70/fc
101/fc
172/ff
3B/d
26D/fc
2B/fc
8A/fc
3A/fc
25C/fc
26B/fc
28C/fc
174A/ff
8G/ff
8G/fc
7A/d
5A/d
18A/d
166A/ff
71/fc
149G/f
149A/f
149H/ff
29
67A/fc
160/ff
173/ff
110/fc
134A/d
152A/ff
147A/d
118A/d
35A/fc
35A/d
83A/fc
114A/d
17A/fc
118B/fc
170/ff
151A/ff
112A/fc
146A/d
148A/d
13B/fc
13B/d
63A/d
165/ff
169/ff
14C/fc
64A/fc
127A/d
82B/d
13A/fc
124F/fc
4X/ff
82A/fc
15B/fc
15A/fc
163A/ff
1D/fc
9A/fc
9A/d
1F/fc
28B/fc

Appendix 10


Similarity
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
L/F
F + PP3
PP1 + PP3
PP1
PP3
L
A
R
V
VP + PP2
Vau
2C/d
2D/d
2A/d
2B/d
31A/d
7A/d
5A/d
18A/d
43A/d
34A/d
34A/fc
174B/ff
28C/fc
174A/ff
2B/fc
25C/fc
26B/fc
7C/fc
18A/fc
8A/fc
8G/ff
8G/fc
1D/d
1A/d
1B/d
1G/d
1E/d
141A/f
149I/ff
149J/ff
149A/f
149H/ff
26D/fc
149G/f
168/ff
167A/ff
70/d
81A/fc
70/fc
101/fc
172/ff
138A/ff
139B/fc
58B/fc
166A/ff
71/fc
158/fc
158/ff
CHR355755/d
159A/d
3B/d
3A/fc
106A/fc
105A/d
105D/d
108A/fc
107A/fc
105A/fc
105D/fc
11A/fc
11A/d
65A/fc
33B/fc
129B/d
66B/d
128A/d
78GI/d
110/fc
67A/fc
160/ff
173/ff
28D/fc
1D/fc
1F/fc
28B/fc
134A/d
146A/d
148A/d
118A/d
152A/ff
151A/ff
164/ff
52B/d
77A/ff
35A/fc
35A/d
14C/fc
127A/d
63A/d
82B/d
114A/d
83A/fc
64A/fc
13A/fc
17A/fc
118B/fc
170/ff
112A/fc
165/ff
169/ff
147A/d
13B/fc
13B/d
4X/ff
15A/fc
163A/ff
15B/fc
82A/fc
124F/fc
29
9A/fc
9A/d

Appendix 10



Appendix 10

Fig. 3 WPGMA phenogram based on Data Set 2.

Appendix 11

Distributions of quantitative and ordered qualitative characters among groups according to the UPGMA phenogram based on Data Set 4 as visualised with box plots.

group 1: 66B/d, 67A/fc, 11A/fc, 64A/fc, 118B/fc, 17A/fc, 112A/fc, 148A/d, 9A/fc, 13A/fc, 13B/fc, 35A/fc, 134A/d
group 2: 8G/fc, 7C/fc, 5A/d, 77A/ff, 34A/fc, 43A/d, 4X/ff, 15A/fc
group 3: 159A/d, 65A/fc, 33B/fc
group 4: 105A/d, 105A/fc, 106A/fc, 108A/fc
group 5: 139B/fc, 58B/fc, 70/fc
group 6: 158/fc, CHR355755/d

1 habit and growth form

group	mean	min	max	var	median
1	2.000	2	2	0.0000000	2.00
2	1.250	1	2	0.2142857	1.00
3	2.000	2	2	0.0000000	2.00
4	2.375	2	3	0.2291667	2.25
5	1.000	1	1	0.0000000	1.00
6	2.000	2	2	0.0000000	2.00

2 plant height

group	mean	min	max	var	median
1	1.666667	1	2	0.2424242	2.0
2	2.000000	2	2	0.0000000	2.0
3	1.333333	1	2	0.3333333	1.0
4	1.500000	1	2	0.3333333	1.5
5	2.666667	2	3	0.3333333	3.0
6	1.000000	1	1	0.0000000	1.0

3 branchlet angle (°)

group	mean	min	max	var	median
1	41.96758	32.0	58.00	51.6985426	42.42857
2	33.51562	26.0	43.00	28.3769531	32.81250
3	46.94444	38.5	56.00	76.8425926	46.33333
4	36.87500	32.5	39.50	11.2291667	37.75000
5	27.43333	26.5	28.00	0.6633333	27.80000
6	52.91667	52.5	53.33	0.3472222	52.91667

4 branchlet diameter (mm)

group	mean	min	max	var	median
1	1.608242	1.2285714	2.228571	0.08595107	1.600000
2	1.410714	1.1714286	1.842857	0.06243440	1.350000
3	1.647619	1.3000000	1.871429	0.09312925	1.771429
4	1.210119	0.7833333	1.400000	0.08548895	1.328571
5	1.547619	1.3000000	1.757143	0.05333333	1.585714
6	1.784286	1.5400000	2.028571	0.11935102	1.784286

5 branchlet indumentum density

group	mean	min	max	var	median
1	2.384615	2	3	0.2564103	2.0
2	2.375000	1	3	0.5535714	2.5
3	4.666667	4	5	0.3333333	5.0
4	4.500000	4	5	0.3333333	4.5
5	3.666667	3	4	0.3333333	4.0
6	4.500000	4	5	0.5000000	4.5

6 branchlet exudate

group	mean	min	max	var	median
1	2.7692308	1	4	1.1923077	3
2	3.5000000	3	5	0.8571429	3
3	0.6666667	0	1	0.3333333	1
4	1.5000000	1	3	1.0000000	1
5	1.0000000	0	2	1.0000000	1
6	1.0000000	1	1	0.0000000	1

9 branchlet glutinous

group	mean	min	max	var	median
1	0.6153846	0	3	1.089744	0
2	0.1250000	0	1	0.125000	0
3	2.0000000	1	3	1.000000	2
4	0.2500000	0	1	0.250000	0
5	1.0000000	1	1	0.000000	1
6	0.0000000	0	0	0.000000	0

10 branchlet spacing

group	mean	min	max	var	median
1	7.134615	4.625000	10.100	3.029223	6.600000
2	10.998214	7.100000	15.100	6.702883	10.700000
3	5.069444	3.666667	5.875	1.486690	5.666667
4	13.833333	11.000000	16.000	4.611111	14.166667
5	11.966667	9.600000	14.900	7.263333	11.400000
6	8.000000	7.000000	9.000	2.000000	8.000000

11 colour of adaxial surface of young leaf (gradient)

group	mean	min	max	var	median
1	2.923077	2	5	0.7435897	3.0
2	3.000000	2	6	2.0000000	2.5
3	2.333333	2	3	0.3333333	2.0
4	2.750000	2	4	0.9166667	2.5
5	2.333333	2	3	0.3333333	2.0
6	3.000000	2	4	2.0000000	3.0

13 colour of adaxial surface of young leaf (depth)

group	mean	min	max	var	median
1	5.230769	4	6	0.6923077	5.0
2	4.875000	4	6	0.6964286	5.0
3	4.000000	3	5	1.0000000	4.0
4	4.500000	3	6	1.6666667	4.5
5	5.000000	5	5	0.0000000	5.0
6	5.500000	5	6	0.5000000	5.5

15 colour of abaxial surface of young leaf (depth)

group	mean	min	max	var	median
1	3.692308	1	6	3.897436	5.0
2	4.750000	3	6	1.357143	4.5
3	1.000000	1	1	0.000000	1.0
4	3.750000	1	6	4.250000	4.0
5	1.000000	1	1	0.000000	1.0
6	1.000000	1	1	0.000000	1.0

17 colour of abaxial surface of young leaf (depth)

group	mean	min	max	var	median
1	2.076923	1	3	0.4102564	2
2	2.500000	2	4	0.5714286	2
3	1.333333	1	2	0.3333333	1
4	1.750000	1	2	0.2500000	2
5	1.000000	1	1	0.0000000	1
6	1.000000	1	1	0.0000000	1

19 lamina orientation (°)

group	mean	min	max	var	median
1	58.20769	40.0	76.0	127.5341	58.00
2	63.50000	52.0	88.0	156.8571	60.00
3	65.83333	54.5	77.5	132.3333	65.50
4	66.00000	52.0	73.0	92.5000	69.50
5	68.00000	66.5	70.0	3.2500	67.50
6	47.25000	46.0	48.5	3.1250	47.25

24 length of lamina (mm)

group	mean	min	max	var	median
1	6.883846	4.66	9.610	2.3356590	6.9400
2	4.586250	3.55	5.950	0.9268268	4.2200
3	8.946667	6.31	11.660	7.1600333	8.8700
4	3.390000	3.06	3.710	0.1138000	3.3950
5	4.213333	3.44	5.680	1.6149333	3.5200
6	11.370500	9.56	13.181	6.5558205	11.3705

25 width of lamina (mm)					
group	mean	min	max	var	median
1	3.090000	2.180000	4.28	0.3845000	3.18
2	1.757500	1.250000	2.34	0.2327357	1.73
3	3.238095	2.514286	3.87	0.4658251	3.33
4	1.722500	1.430000	1.98	0.0550916	1.74
5	1.586667	1.280000	1.95	0.1146333	1.53
6	3.480000	2.680000	4.28	1.2800000	3.48

26 ratio length/width					
group	mean	min	max	var	median
1	2.241693	1.919189	2.723493	0.0539207	2.264752
2	2.758772	1.972839	4.596569	0.6533027	2.674770
3	2.744679	2.492765	3.027986	0.0723545	2.713286
4	1.987820	1.841222	2.222439	0.0337531	1.943810
5	2.645836	2.299556	2.943282	0.1053844	2.694669
6	3.366077	3.115991	3.616163	0.1250858	3.366077

27 margin recurved					
group	mean	min	max	var	median
1	1.230769	1	2	0.1923077	1.0
2	2.750000	1	4	1.3571429	3.0
3	2.000000	2	2	0.0000000	2.0
4	2.750000	2	3	0.2500000	3.0
5	3.333333	3	4	0.3333333	3.0
6	3.500000	3	4	0.5000000	3.5

28 midvein evident					
group	mean	min	max	var	median
1	1.230769	1	2	0.1923077	1.0
2	2.750000	1	4	0.7857143	3.0
3	2.333333	2	3	0.3333333	2.0
4	3.500000	3	4	0.3333333	3.5
5	3.666667	3	4	0.3333333	4.0
6	3.500000	3	4	0.5000000	3.5

29 density of trichomes on adaxial surface of young leaf					
group	mean	min	max	var	median
1	1.230769	1	2	0.1923077	1
2	0.750000	0	1	0.2142857	1
3	3.000000	3	3	0.0000000	3
4	2.000000	1	3	0.6666667	2
5	1.666667	1	2	0.3333333	2
6	3.000000	3	3	0.0000000	3

30 indumentum density of abaxial surface of young leaf					
group	mean	min	max	var	median
1	2.076923	1	3	0.4102564	2
2	2.875000	2	4	0.6964286	3
3	3.666667	3	4	0.3333333	4
4	3.750000	3	4	0.2500000	4
5	4.000000	4	4	0.0000000	4
6	5.000000	5	5	0.0000000	5

31 density of trichomes on adaxial surface of old leaf					
group	mean	min	max	var	median
1	1.230769	1	2	0.1923077	1
2	0.750000	0	1	0.2142857	1
3	3.000000	3	3	0.0000000	3
4	2.000000	1	3	0.6666667	2
5	1.666667	1	2	0.3333333	2
6	3.000000	3	3	0.0000000	3

32 indumentum density of abaxial surface of old leaf					
group	mean	min	max	var	median
1	2.076923	1	3	0.4102564	2
2	2.875000	2	4	0.6964286	3
3	3.666667	3	4	0.3333333	4
4	3.750000	3	4	0.2500000	4
5	4.000000	4	4	0.0000000	4
6	5.000000	5	5	0.0000000	5

33 exudate colour on abaxial surface of young leaf (gradient)					
group	mean	min	max	var	median
1	3.8461538	0	5	2.1410256	4.0
2	4.3750000	4	5	0.2678571	4.0
3	0.0000000	0	0	0.0000000	0.0
4	1.2500000	0	5	6.2500000	0.0
5	0.3333333	0	1	0.3333333	0.0
6	1.5000000	0	3	4.5000000	1.5

34 exudate colour on adaxial surface of lamina of young leaf (depth)					
group	mean	min	max	var	median
1	1.3846154	0	2	0.4230769	1.0
2	1.3750000	1	2	0.2678571	1.0
3	0.0000000	0	0	0.0000000	0.0
4	0.5000000	0	2	1.0000000	0.0
5	0.3333333	0	1	0.3333333	0.0
6	0.5000000	0	1	0.5000000	0.5

35 exudate density on adaxial surface of lamina of young leaf					
group	mean	min	max	var	median
1	1.1538462	0	2	0.3076923	1
2	1.0000000	1	1	0.0000000	1
3	0.0000000	0	0	0.0000000	0
4	0.2500000	0	1	0.2500000	0
5	0.3333333	0	1	0.3333333	0
6	1.0000000	1	1	NA	1

36 exudate colour on abaxial surface of lamina of young leaf (gradient)					
group	mean	min	max	var	median
1	4.6153846	3	5	0.5897436	5.0
2	4.8750000	4	7	1.8392857	4.0
3	2.3333333	1	3	1.3333333	3.0
4	5.0000000	5	5	0.0000000	5.0
5	0.3333333	0	1	0.3333333	0.0
6	4.5000000	4	5	0.5000000	4.5

37 exudate colour on abaxial surface of lamina of young leaf (depth)					
group	mean	min	max	var	median
1	1.9230769	1	2	0.0769230	2
2	1.8750000	1	3	0.4107142	2
3	1.0000000	1	1	0.0000000	1
4	1.2500000	1	2	0.2500000	1
5	0.3333333	0	1	0.3333333	0
6	1.0000000	1	1	0.0000000	1

38 exudate density abaxial surface of lamina of young leaf					
group	mean	min	max	var	median
1	2.3846154	1	3	0.7564103	3.0
2	3.0000000	2	4	0.2857143	3.0
3	1.0000000	1	1	0.0000000	1.0
4	2.0000000	1	3	0.6666667	2.0
5	0.3333333	0	1	0.3333333	0.0
6	1.5000000	1	2	0.5000000	1.5

41 exudate density on adaxial surface of lamina of old leaf					
group	mean	min	max	var	median
1	1.0769231	0	2	0.2435897	1.0
2	1.0000000	1	1	0.0000000	1.0
3	0.3333333	0	1	0.3333333	0.0
4	0.2500000	0	1	0.2500000	0.0
5	0.3333333	0	1	0.3333333	0.0
6	0.5000000	0	1	0.5000000	0.5

44 exudate density on abaxial surface of lamina of old leaf					
group	mean	min	max	var	median
1	2.3846154	1	3	0.5897436	3.0
2	3.0000000	2	4	0.2857143	3.0
3	1.0000000	1	1	0.0000000	1.0
4	2.0000000	1	3	0.6666667	2.0
5	0.3333333	0	1	0.3333333	0.0
6	1.5000000	1	2	0.5000000	1.5

45 leaves glutinous					
group	mean	min	max	var	median
1	0.8461538	0	3	1.3076923	0.0
2	0.1250000	0	1	0.1250000	0.0
3	2.3333333	1	3	1.3333333	3.0
4	0.5000000	0	1	0.3333333	0.5
5	1.0000000	1	1	0.0000000	1.0
6	0.0000000	0	0	0.0000000	0.0

47 leaf spacing					
group	mean	min	max	var	median
1	8.115385	4.1	15.0	13.696410	7.10
2	8.412500	5.0	12.2	7.298393	8.15
3	6.933333	4.2	11.5	15.843333	5.10
4	11.600000	8.2	18.0	19.246667	10.10
5	8.833333	7.5	10.7	2.773333	8.30
6	7.100000	6.0	8.2	2.420000	7.10>

48 compactness of panicle					
group	mean	min	max	var	median
1	3.076923	3	4	0.0769230	3.0
2	2.250000	2	3	0.2142857	2.0
3	2.500000	2	3	0.5000000	2.5
4	3.000000	3	3	0.0000000	3.0
5	2.000000	2	2	0.0000000	2.0
6	3.000000	3	3	NA	3.0

49 number of capitula per panicle					
group	mean	min	max	var	median
1	47.69231	14.50000	111.1667	973.28669	33.00000
2	102.23155	17.28571	301.8333	8265.8828	71.00000
3	108.25000	34.50000	182.0000	10878.125	108.25000
4	8.96250	6.00000	11.6000	6.105625	9.12500
5	123.20952	54.40000	256.8000	13388.868	58.42857
6	11.00000	11.00000	11.0000	NA	11.00000

50 diameter of panicle (mm)					
group	mean	min	max	var	median
1	25.01172	15.00000	41.8	78.39504	19.80000
2	26.19821	9.00000	40.5	103.67268	25.80000
3	45.00000	32.00000	58.0	338.00000	45.00000
4	14.74167	10.20000	19.6	17.20917	14.58333
5	31.97778	23.33333	45.8	146.29481	26.80000
6	12.50000	12.50000	12.5	NA	12.50000

51 50: (49x57)					
group	mean	min	max	var	median
1	0.3006614	0.1463554	0.6496881	0.0156262	0.2671262
2	0.2407494	0.1056536	0.3359104	0.0057602	0.2456085
3	0.2338033	0.1410094	0.3265973	0.0172214	0.2338033
4	0.5411403	0.5030488	0.5762712	0.0013469	0.5441008
5	0.1978318	0.1371915	0.2438847	0.0030054	0.2124192
6	0.6209637	0.6209637	0.6209637	NA	0.6209637

52 structure of panicle					
group	mean	min	max	var	median
1	2.384615	2.0	3.0	0.2147436	2.00
2	3.187500	2.5	4.0	0.2098214	3.00
3	2.750000	2.0	3.5	1.1250000	2.75
4	1.666667	1.0	2.0	0.3333333	2.00
5	3.333333	2.5	4.5	1.0833333	3.00
6	1.500000	1.5	1.5	NA	1.50

53 length of pedicel (mm)					
group	mean	min	max	var	median
1	1.253846	0.37	3.22	0.6262756	1.030
2	1.376250	0.63	2.19	0.2419982	1.405
3	3.900000	1.97	5.83	7.4498000	3.900
4	3.295000	1.91	5.72	2.8687000	2.775
5	2.153333	1.72	2.66	0.2249333	2.080
6	1.270000	1.27	1.27	NA	1.270

54 top width of capitulum (mm)					
group	mean	min	max	var	median
1	3.214615	2.39	4.05	0.2316436	3.20
2	2.306250	1.79	2.89	0.1322268	2.29
3	3.810000	3.59	4.03	0.0968000	3.81
4	3.996667	3.38	4.31	0.2852333	4.30
5	2.460000	1.87	2.77	0.2613000	2.74
6	2.860000	2.86	2.86	NA	2.86

55 length of capitulum (mm)					
group	mean	min	max	var	median
1	5.778462	5.06	6.60	0.1832141	5.640
2	4.802500	4.35	5.82	0.2251928	4.705
3	5.740000	5.56	5.92	0.0648000	5.740
4	7.376667	7.24	7.45	0.0140333	7.440
5	5.420000	5.13	5.68	0.0763000	5.450
6	6.330000	6.33	6.33	NA	6.330

56 involucre length (mm)					
group	mean	min	max	var	median
1	4.753846	4.13	5.92	0.2806256	4.79
2	3.867500	3.30	4.73	0.1990786	3.77
3	4.660000	4.63	4.69	0.0018000	4.66
4	5.606667	5.19	6.25	0.3194333	5.38
5	4.420000	4.30	4.56	0.0172000	4.40
6	4.860000	4.86	4.86	NA	4.86

57 body width of capitulum (mm)					
group	mean	min	max	var	median
1	2.106154	1.70	2.49	0.0601089	2.100
2	1.455000	0.96	1.94	0.0914571	1.445
3	2.550000	2.26	2.84	0.1682000	2.550
4	3.020000	2.91	3.20	0.0247000	2.950
5	1.733333	1.30	2.02	0.1457333	1.880
6	1.830000	1.83	1.83	NA	1.830

58 ratio 57:56					
group	mean	min	max	var	median
1	0.3667138	0.3046309	0.4847978	0.0027855	0.3728064
2	0.3048443	0.1903170	0.3855330	0.0036713	0.3156213
3	0.4433732	0.4070359	0.4797104	0.0026407	0.4433732
4	0.4096108	0.3902609	0.4418255	0.0007888	0.3967462
5	0.3179384	0.2530982	0.3556113	0.0031807	0.3451056
6	0.2897346	0.2897346	0.2897346	NA	0.2897346

59 ratio 54:57					
group	mean	min	max	var	median
1	1.537579	1.145238	1.800418	0.0438185	1.603390
2	1.621429	1.334763	1.996667	0.0653172	1.536267
3	1.527324	1.266630	1.788017	0.1359219	1.527324
4	1.325714	1.147703	1.482037	0.0282976	1.347400
5	1.421328	1.371762	1.458099	0.0019862	1.434121
6	1.576186	1.576186	1.576186	NA	1.576186

60 floret number of capitulum					
group	mean	min	max	var	median
1	13.353846	9.5	19.8	10.09603	12.90
2	7.312500	5.0	8.7	1.46125	7.65
3	17.050000	16.3	17.8	1.12500	17.05
4	16.833333	12.9	18.8	11.60333	18.80
5	9.966667	5.2	13.6	18.60333	11.10
6	5.500000	5.5	5.5	NA	5.50

61 corolla tube length (mm)					
group	mean	min	max	var	median
1	2.770758	2.2375	3.2600	0.0724868	2.767000
2	2.388354	2.0575	2.7750	0.0659478	2.357917
3	2.951250	2.9450	2.9575	0.0000781	2.951250
4	3.663167	3.4615	3.9160	0.0536060	3.612000
5	2.505000	2.4550	2.5350	0.0019000	2.525000
6	2.900000	2.9000	2.9000	NA	2.900000

62 corolla lobe length (mm)					
group	mean	min	max	var	median
1	0.6012857	0.3900000	0.7857143	0.0086336	0.6225
2	0.5736458	0.5166667	0.7100000	0.0042581	0.5500
3	0.5600000	0.5150000	0.6050000	0.0040500	0.5600
4	0.7905000	0.7825000	0.8015000	0.0000970	0.7875
5	0.6733333	0.5425000	0.8900000	0.0357145	0.5875
6	0.6125000	0.6125000	0.6125000	NA	0.6125

63 corolla lobe width (mm)					
group	mean	min	max	var	median
1	0.3290495	0.2550	0.3900	0.0017966	0.32000
2	0.3150000	0.2650	0.3950	0.0025089	0.29500
3	0.3362500	0.2850	0.3875	0.0052531	0.33625
4	0.4450000	0.3925	0.4975	0.0027562	0.44500
5	0.3350000	0.2875	0.3650	0.0017312	0.35250
6	0.3225000	0.3225	0.3225	NA	0.32250

64 diameter of corolla (mm)					
group	mean	min	max	var	median
1	0.9705604	0.6880	1.414286	0.0402258	0.9200000
2	0.8614167	0.6500	1.085000	0.0227876	0.8569167
3	1.0012500	0.9375	1.065000	0.0081281	1.0012500
4	1.4631667	1.4095	1.525000	0.0033850	1.4550000
5	1.1133333	0.9550	1.385000	0.0558583	1.0000000
6	0.9900000	0.9900	0.990000	NA	0.9900000

65 style length (mm)					
group	mean	min	max	var	median
1	2.498879	1.8600	2.8100	0.0639522	2.522500
2	2.107500	1.6950	2.5500	0.1168553	2.067500
3	2.535000	2.3975	2.6725	0.0378125	2.535000
4	2.997333	2.8275	3.1145	0.0226725	3.050000
5	2.161019	2.0450	2.2575	0.0115753	2.180556
6	2.550000	2.5500	2.5500	NA	2.550000

66 stigmatic lobe length (mm)					
group	mean	min	max	var	median
1	0.6411484	0.5375	0.8464286	0.0084581	0.65100
2	0.6753125	0.5000	0.9250000	0.0256043	0.61750
3	0.7162500	0.5675	0.8650000	0.0442531	0.71625
4	1.0836667	1.0725	1.0900000	0.0000940	1.08850
5	0.7840333	0.7000	0.9021000	0.0110798	0.75000
6	0.8800000	0.8800	0.8800000	NA	0.88000

67 outer involucral bracts shape					
group	mean	min	max	var	median
1	2.653846	2.0	3.0	0.2243589	3.00
2	1.375000	1.0	2.0	0.2678571	1.00
3	3.000000	3.0	3.0	0.0000000	3.00
4	1.750000	1.5	2.0	0.1250000	1.75
5	1.166667	1.0	1.5	0.0833333	1.00
6	3.000000	3.0	3.0	NA	3.00

69 outer involucral bracts spreading					
group	mean	min	max	var	median
1	0.4615385	0	1	0.2692308	0.0
2	0.0000000	0	0	0.0000000	0.0
3	0.5000000	0	1	0.5000000	0.5
4	0.0000000	0	0	0.0000000	0.0
5	0.0000000	0	0	0.0000000	0.0
6	0.0000000	0	0	NA	0.0

70 density of trichomes on abaxial surface of outer involucral bracts					
group	mean	min	max	var	median
1	1.923077	1	3	0.5769231	2
2	1.250000	1	2	0.2142857	1
3	3.000000	3	3	0.0000000	3
4	3.000000	3	3	0.0000000	3
5	1.000000	1	1	0.0000000	1
6	3.000000	3	3	NA	3

71 number of outer involucral bracts					
group	mean	min	max	var	median
1	11.20000	8.7	14.0	2.881667	10.70
2	8.12500	6.9	9.7	1.050714	8.05
3	9.60000	9.2	10.0	0.320000	9.60
4	18.73333	16.9	20.0	2.643333	19.30
5	13.26667	10.8	15.8	6.253333	13.20
6	9.80000	9.8	9.8	NA	9.80

72 inner involucral bracts length (mm)					
group	mean	min	max	var	median
1	4.246923	3.84	4.660000	0.0599397	4.230
2	3.623750	3.11	4.380000	0.1723982	3.660
3	4.155000	3.91	4.400000	0.1200500	4.155
4	5.056667	4.80	5.266667	0.0386888	5.080
5	3.886667	3.69	4.170000	0.0632333	3.800
6	4.440000	4.44	4.440000	NA	4.440

73 inner involucral bracts width (mm)					
group	mean	min	max	var	median
1	0.8846923	0.7300000	1.00	0.0061878	0.880
2	0.7450000	0.5000000	1.06	0.0297714	0.765
3	1.0550000	0.9900000	1.12	0.0084500	1.055
4	1.0783333	0.9333333	1.14	0.0097000	1.120
5	0.7766667	0.7300000	0.80	0.0016333	0.800
6	0.7000000	0.7000000	0.70	NA	0.700

74 ratio 72:73					
group	mean	min	max	var	median
1	4.979882	4.338532	6.555476	0.3460668	4.862546
2	5.249620	4.177121	7.941667	1.6679292	4.889048
3	4.064850	3.658792	4.470909	0.3297672	4.064850
4	4.503866	4.268257	4.651964	0.0425515	4.591378
5	5.213712	4.783651	5.496890	0.1433586	5.360595
6	6.875595	6.875595	6.875595	NA	6.875595

75 inner involucral bracts apex spreading					
group	mean	min	max	var	median
1	2.846154	2	3	0.1410256	3.0
2	2.625000	2	3	0.2678571	3.0
3	2.500000	2	3	0.5000000	2.5
4	3.000000	3	3	0.0000000	3.0
5	2.666667	2	3	0.3333333	3.0
6	3.000000	3	3	NA	3.0

76 density of trichomes on abaxial surface of inner involucral bracts					
group	mean	min	max	var	median
1	1.00	0	2	0.5000000	1.0
2	0.25	0	1	0.2142857	0.0
3	1.50	1	2	0.5000000	1.5
4	1.00	1	1	0.0000000	1.0
5	0.00	0	0	0.0000000	0.0
6	1.00	1	1	NA	1.0

77 number of inner involucral bracts					
group	mean	min	max	var	median
1	9.000000	5.8	14.8	5.5483333	8.3
2	6.925000	5.5	8.1	0.9192857	6.8
3	12.500000	9.6	15.4	16.820000	12.5
4	6.700000	6.2	7.3	0.3100000	6.6
5	6.666667	4.6	9.0	4.8933333	6.4
6	8.700000	8.7	8.7	NA	8.7

78 total number of involucral bracts					
group	mean	min	max	var	median
1	20.11538	16.3	24.8	6.159744	19.70
2	14.97500	13.4	17.5	2.516429	14.80
3	21.85000	18.6	25.1	21.125000	21.85
4	25.10000	24.3	26.0	0.730000	25.00
5	20.03333	15.3	24.8	22.563333	20.00
6	18.20000	18.2	18.2	NA	18.20

79 number of receptacle scales per capitulum					
group	mean	min	max	var	median
1	8.807692	2.9	16.5	22.412436	8.10
2	4.762500	1.3	13.4	16.562679	3.05
3	13.150000	10.3	16.0	16.245000	13.15
4	24.166667	21.7	26.9	6.813333	23.90
5	7.666667	2.8	10.7	18.123333	9.50
6	0.000000	0.0	0.0	NA	0.00

83 total number of white tipped appendages					
group	mean	min	max	var	median
1	17.82308	11.4	25.3	25.26359	17.20
2	11.66250	7.5	21.5	22.11411	10.05
3	25.65000	19.9	31.4	66.12500	25.65
4	30.86667	27.9	34.2	10.02333	30.50
5	14.33333	7.4	19.7	39.66333	15.90
6	8.70000	8.7	8.7	NA	8.70

84 pappus length (mm)					
group	mean	min	max	var	median
1	3.158462	2.82	3.54	0.0656641	3.160
2	2.781250	2.42	3.56	0.1339267	2.765
3	3.100000	3.03	3.17	0.0098000	3.100
4	3.845000	3.58	4.03	0.0359000	3.885
5	2.826667	2.55	2.97	0.0574333	2.960
6	3.020000	3.02	3.02	NA	3.020

85 pappus thickening					
group	mean	min	max	var	median
1	2.038462	1.0	3	0.6442308	2
2	1.937500	1.0	3	0.3169643	2
3	1.000000	1.0	1	0.0000000	1
4	3.000000	3.0	3	0.0000000	3
5	2.166667	1.5	3	0.5833333	2
6	2.000000	2.0	2	NA	2

86 achenes length (mm)					
group	mean	min	max	var	median
1	1.0534615	0.6400	1.592500	0.0953943	1.00750
2	1.0553125	0.7900	1.187500	0.0233132	1.10875
3	1.5525000	1.5500	1.555000	0.0000125	1.55250
4	1.2229167	0.9575	1.526667	0.0600326	1.20375
5	0.9516667	0.8625	1.110000	0.0189020	0.88250
6	1.2125000	1.2125	1.212500	NA	1.21250

87 achene width (mm)					
group	mean	min	max	var	median
1	0.4531923	0.3000	0.5550000	0.0043094	0.46000
2	0.4318000	0.3250	0.5204000	0.0061653	0.46125
3	0.5850000	0.5700	0.6000000	0.0004500	0.58500
4	0.4677083	0.4125	0.5533333	0.0037640	0.45250
5	0.3591667	0.3125	0.3925000	0.0017333	0.37250
6	0.5675000	0.5675	0.5675000	NA	0.56750

88 ratio 86:87					
group	mean	min	max	var	median
1	2.313358	1.745221	3.182444	0.1928994	2.201643
2	2.482174	2.217258	3.010182	0.0626165	2.414694
3	2.666088	2.597950	2.734225	0.0092854	2.666088
4	2.612013	2.343651	2.787650	0.0433747	2.658375
5	2.665943	2.330407	2.847422	0.0846262	2.820000
6	2.129947	2.129947	2.129947	NA	2.129947

89 density of achenial hair					
group	mean	min	max	var	median
1	1.6923077	0.5	3.0	1.0224359	1
2	1.6875000	0.0	3.0	1.2098214	2
3	2.0000000	0.0	4.0	8.0000000	2
4	0.0000000	0.0	0.0	0.0000000	0
5	0.1666667	0.0	0.5	0.0833333	0
6	4.0000000	4.0	4.0	NA	4

90 glandular hair on achenes					
group	mean	min	max	var	median
1	0.4615385	0	1.0	0.1858974	0.50
2	0.8750000	0	3.0	0.9107142	0.75
3	0.5000000	0	1.0	0.5000000	0.50
4	1.0000000	1	1.0	0.0000000	1.00
5	0.3333333	0	0.5	0.0833333	0.50
6	2.0000000	2	2.0	NA	2.00

Distributions of quantitative and ordered qualitative Microcharacters among groups according to the UPGMA phenogram based on Data Set 6 as visualised with box plots.

group 1: 8G/fc, 7C/fc, 77A/ff, 34A/fc, 43A/d
group 2: 11A/fc, 64A/fc, 118B/fc, 17A/fc, 112A/fc, 148A/d, 9A/fc, 13A/fc, 15A/fc, 35A/fc
group 3: 65A/fc 33B/fc
group 4: 139B/fc, 58B/fc, 70/fc
group 5: 105A/fc, 106A/fc
group 6: 158/fc

MC1 pappus breadth at tip (µm)					
group	mean	min	max	var	median
1	52.50000	42.50	60.00	43.78125	53.750
2	68.37500	61.75	74.50	23.50347	68.750
3	62.12500	60.50	63.75	5.28125	62.125
4	52.91667	45.75	64.00	94.77083	49.000
5	49.62500	49.00	50.25	0.78125	49.625
6	88.50000	88.50	88.50	NA	88.500

MC2 number of apical cells					
group	mean	min	max	var	median
1	3.960000	3.6	4.2	0.0830000	4.10
2	4.070000	3.0	5.2	0.4556666	4.30
3	4.200000	3.8	4.6	0.3200000	4.20
4	3.833333	3.6	4.0	0.0433333	3.90
5	4.050000	3.9	4.2	0.0450000	4.05
6	5.200000	5.2	5.2	NA	5.20

MC3 breadth of apical cells ((µm)					
group	mean	min	max	var	median
1	22.41667	18.95833	24.54167	4.423611	22.62500
2	29.97083	22.25000	34.58333	18.230729	30.43750
3	26.81250	24.25000	29.37500	13.132812	26.81250
4	22.09722	15.87500	27.50000	34.288773	22.91667
5	20.66667	20.16667	21.16667	0.500000	20.66667
6	32.12500	32.12500	32.12500	NA	32.12500

MC4 pappus breadth at widest part (µm)					
group	mean	min	max	var	median
1	71.60000	65.25	78.75	23.45625	71.000
2	86.05000	72.75	101.75	104.96944	85.000
3	71.87500	70.00	73.75	7.03125	71.875
4	75.41667	65.25	86.00	107.77083	75.000
5	87.25000	87.25	87.25	0.00000	87.250
6	97.50000	97.50	97.50	NA	97.500

MC5 pappus breadth at the centre (µm)					
group	mean	min	max	var	median
1	21.45000	19.25	23.75	3.6062500	22.00
2	24.07500	18.75	27.25	7.8618056	24.25
3	24.75000	24.25	25.25	0.5000000	24.75
4	20.66667	20.25	21.50	0.5208333	20.25
5	21.50000	20.00	23.00	4.5000000	21.50
6	26.75000	26.75	26.75	NA	26.75

MC6 length of barbellae (µm)					
group	mean	min	max	var	median
1	24.26667	18.58333	30.33333	28.421528	24.50000
2	39.64167	26.91667	58.41667	133.22075	35.45833
3	24.33333	22.75000	25.91667	5.013889	24.33333
4	28.00000	23.25000	35.25000	40.687500	25.50000
5	33.25000	28.75000	37.75000	40.500000	33.25000
6	49.66667	49.66667	49.66667	NA	49.66667

MC7 distance between barbellae and axis (µm)					
group	mean	min	max	var	median
1	12.50000	10.00000	13.83333	3.1284722	13.66667
2	16.60000	12.00000	21.41667	8.6169753	16.54167
3	13.45833	12.50000	14.41667	1.8368055	13.45833
4	12.08333	10.25000	15.16667	7.2152777	10.83333
5	12.12500	11.91667	12.33333	0.0868055	12.12500
6	22.41667	22.41667	22.41667	NA	22.41667

MC8 density of the twin hairs on achene					
group	mean	min	max	var	median
1	1.7000000	0.0	3.5	2.7000000	2.00
2	2.1000000	0.5	4.0	1.4333333	2.25
3	1.7500000	0.0	3.5	6.1250000	1.75
4	0.3333333	0.0	0.5	0.0833333	0.50
5	0.2500000	0.0	0.5	0.1250000	0.25
6	4.5000000	4.5	4.5	NA	4.50

MC9 density of papillae on achene					
group	mean	min	max	var	median
1	1.2000000	0.0	4.0	2.5750000	0.50
2	0.4500000	0.0	2.0	0.4138888	0.25
3	0.5000000	0.0	1.0	0.5000000	0.50
4	0.6666667	0.5	1.0	0.0833333	0.50
5	0.7500000	0.0	1.5	1.1250000	0.75
6	2.5000000	2.5	2.5	NA	2.50

MC10 anther insertion point in corolla (mm)					
group	mean	min	max	var	median
1	0.86700	0.7600	0.9850	0.0063575	0.86500
2	0.90575	0.5325	1.2125	0.0310361	0.92000
3	0.89875	0.8625	0.9350	0.0026281	0.89875
4	0.92500	0.8525	0.9725	0.0040687	0.95000
5	1.31500	1.2350	1.3950	0.0128000	1.31500
6	0.96500	0.9650	0.9650	NA	0.96500

MC11 corolla tube length (mm)					
group	mean	min	max	var	median
1	2.604000	2.23	2.85	0.0555800	2.660
2	2.683000	2.14	3.12	0.1201788	2.665
3	2.900000	2.88	2.92	0.0008000	2.900
4	2.536667	2.51	2.58	0.0014333	2.520
5	3.760000	3.58	3.94	0.0648000	3.760
6	2.660000	2.66	2.66	NA	2.660

MC12 ratio MC11:MC10					
group	mean	min	max	var	median
1	3.021377	2.906390	3.160736	0.0133215	2.955825
2	3.063159	2.423934	4.436853	0.3421681	2.982241
3	3.249159	3.130969	3.367349	0.0279378	3.249159
4	2.764344	2.653913	2.965748	0.0305171	2.673371
5	2.876350	2.824926	2.927773	0.0052886	2.876350
6	2.782660	2.782660	2.782660	NA	2.782660

MC13 anther connective base length (µm)					
group	mean	min	max	var	median
1	23.20	20.0	26.9	6.40500	23.50
2	24.33	15.3	34.0	30.27344	24.60
3	19.65	19.1	20.2	0.60500	19.65
4	19.50	17.3	22.6	7.63000	18.60
5	28.75	26.4	31.1	11.04500	28.75
6	30.40	30.4	30.4	NA	30.40

MC14 anther basal appendages length (µm)					
group	mean	min	max	var	median
1	32.58000	25.9	38.7	26.367000	31.00
2	39.20000	32.2	53.7	44.242222	37.20
3	26.95000	26.5	27.4	0.405000	26.95
4	32.86667	30.2	35.3	6.543333	33.10
5	37.55000	35.3	39.8	10.125000	37.55
6	38.30000	38.3	38.3	NA	38.30

MC15 ratio MC13:MC14					
group	mean	min	max	var	median
1	0.7216148	0.6023843	0.7757229	0.0054996	0.7640776
2	0.6232609	0.4030816	0.7355642	0.0082895	0.6285550
3	0.7302461	0.7225923	0.7378998	0.0001171	0.7302461
4	0.5949433	0.5305110	0.6816572	0.0060836	0.5726619
5	0.7735611	0.6666798	0.8804423	0.0228472	0.7735611
6	0.8561524	0.8561524	0.8561524	NA	0.8561524

Appendix 12

Polyacrylamide gels of amplified fragment length polymorphisms.

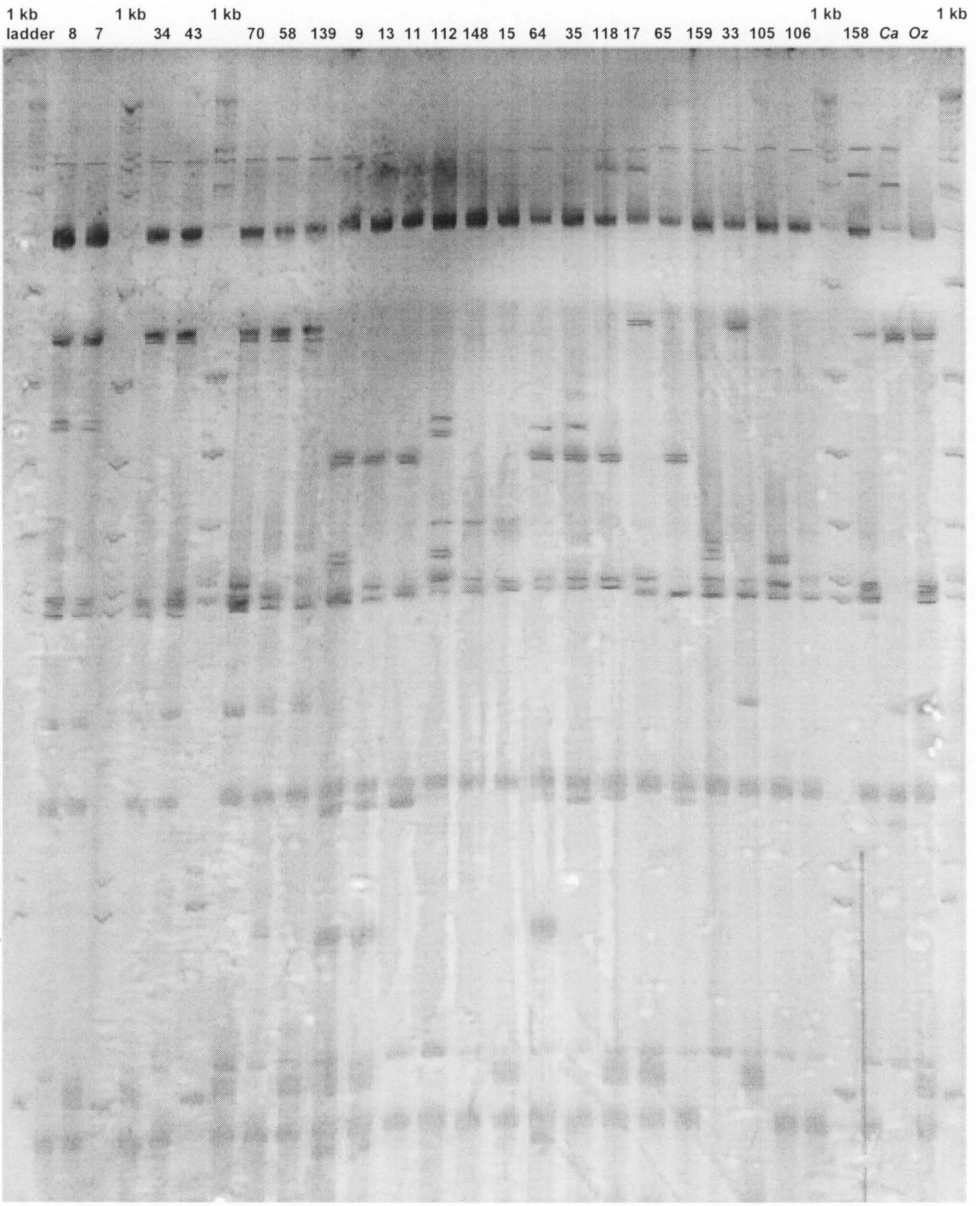


Fig. 1 AFLP fingerprints of genomic DNA of 25 representatives of distinct *Ozothamnus leptophyllus* populations, *Cassinia aculeata* (*Ca*) and *Ozothamnus rodwayi* (*Oz*) using the primer combination PstI+ACC/MseI+CAG.

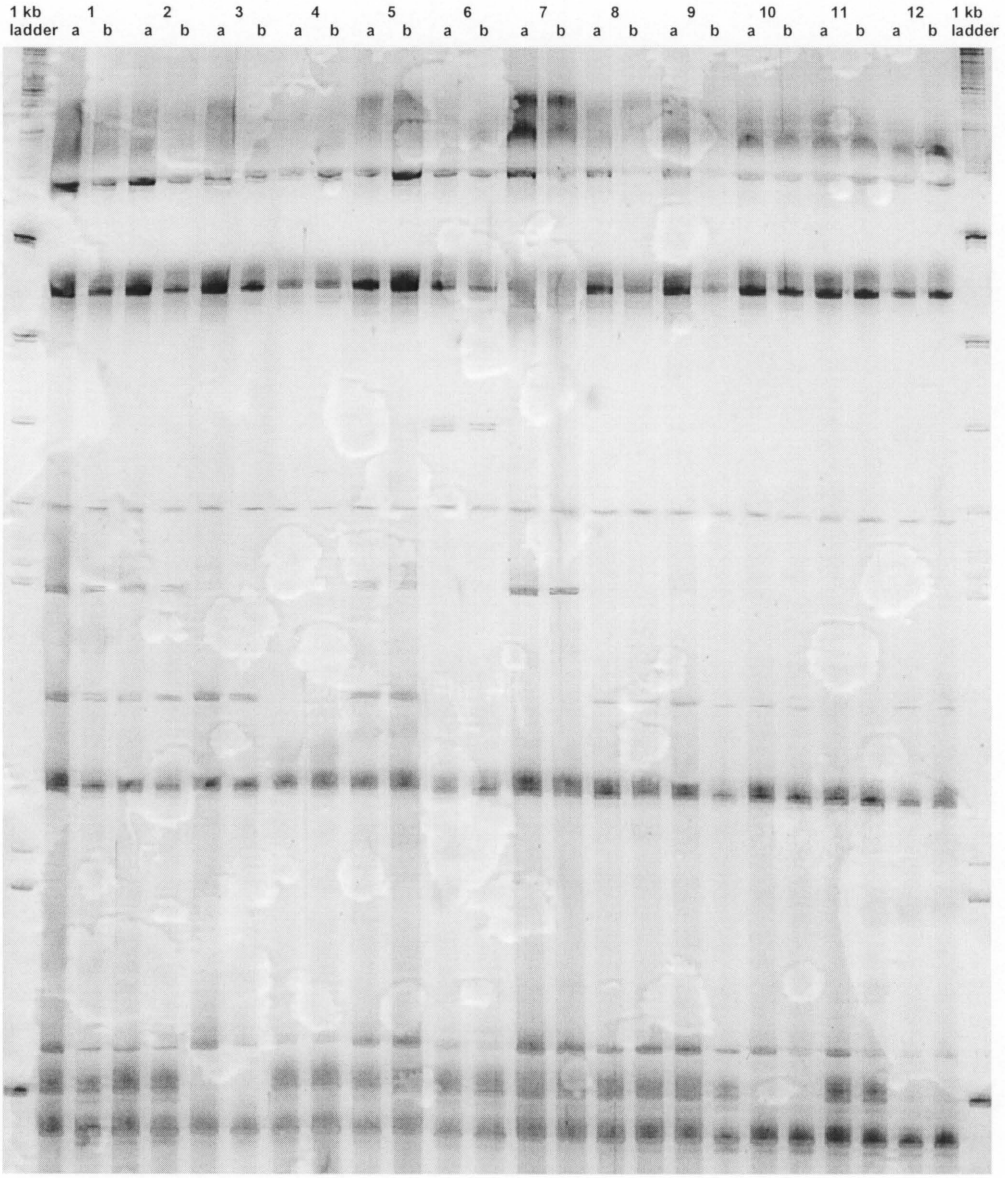


Fig. 2 AFLP fingerprints of genomic DNA of 12 *Ozothamnus* specimens from the Cass population using the primer combination PstI+ACC/MseI+CAG. Each of the 12 specimens is represented by 2 samples (a and b) derived from 2 separate extractions carried through the complete AFLP process.

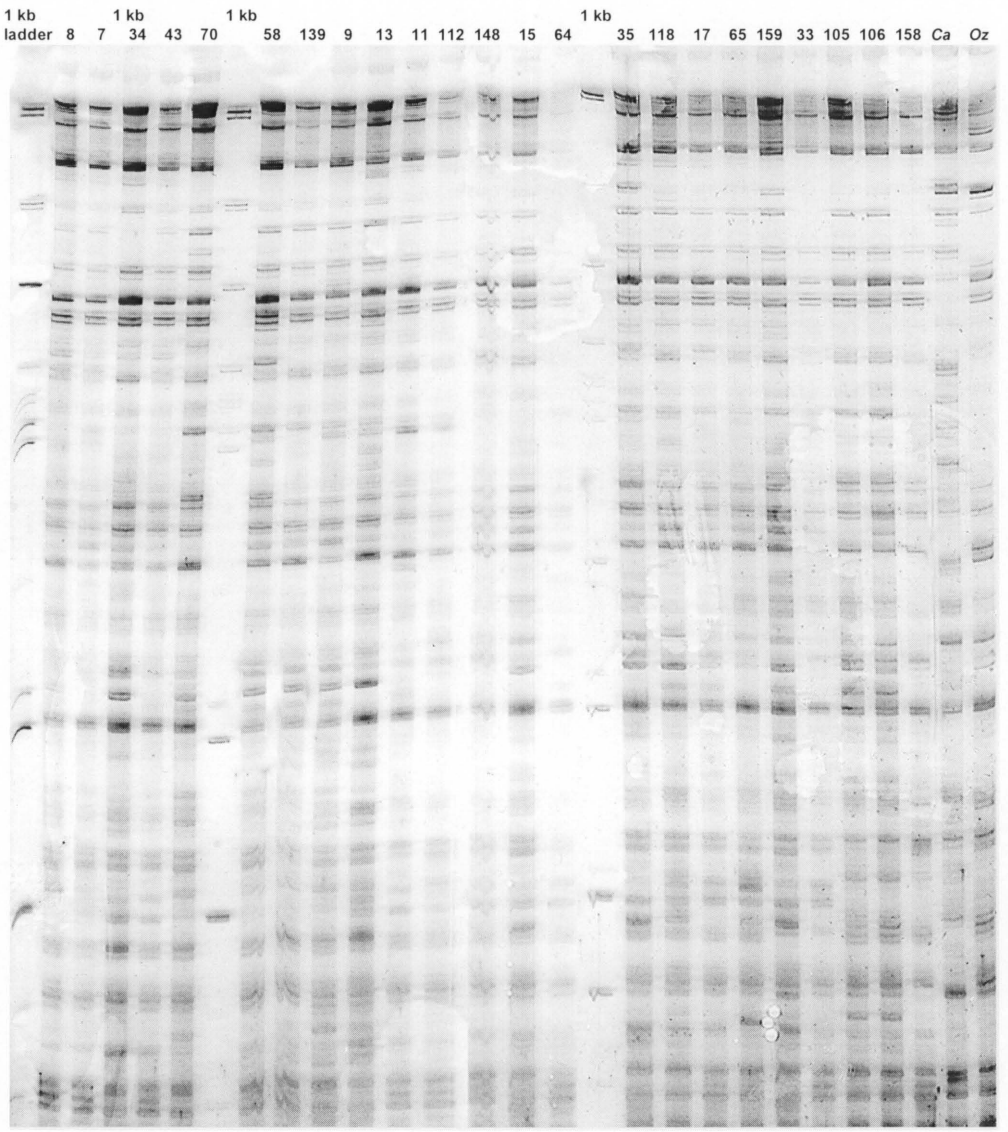


Fig. 3 AFLP fingerprints of genomic DNA of 25 representatives of distinct *Ozothamnus leptophyllus* populations, *Cassinia aculeata* (*Ca*) and *Ozothamnus rodwayi* (*Oz*) using the primer combination PstI+AC/MseI+CA.

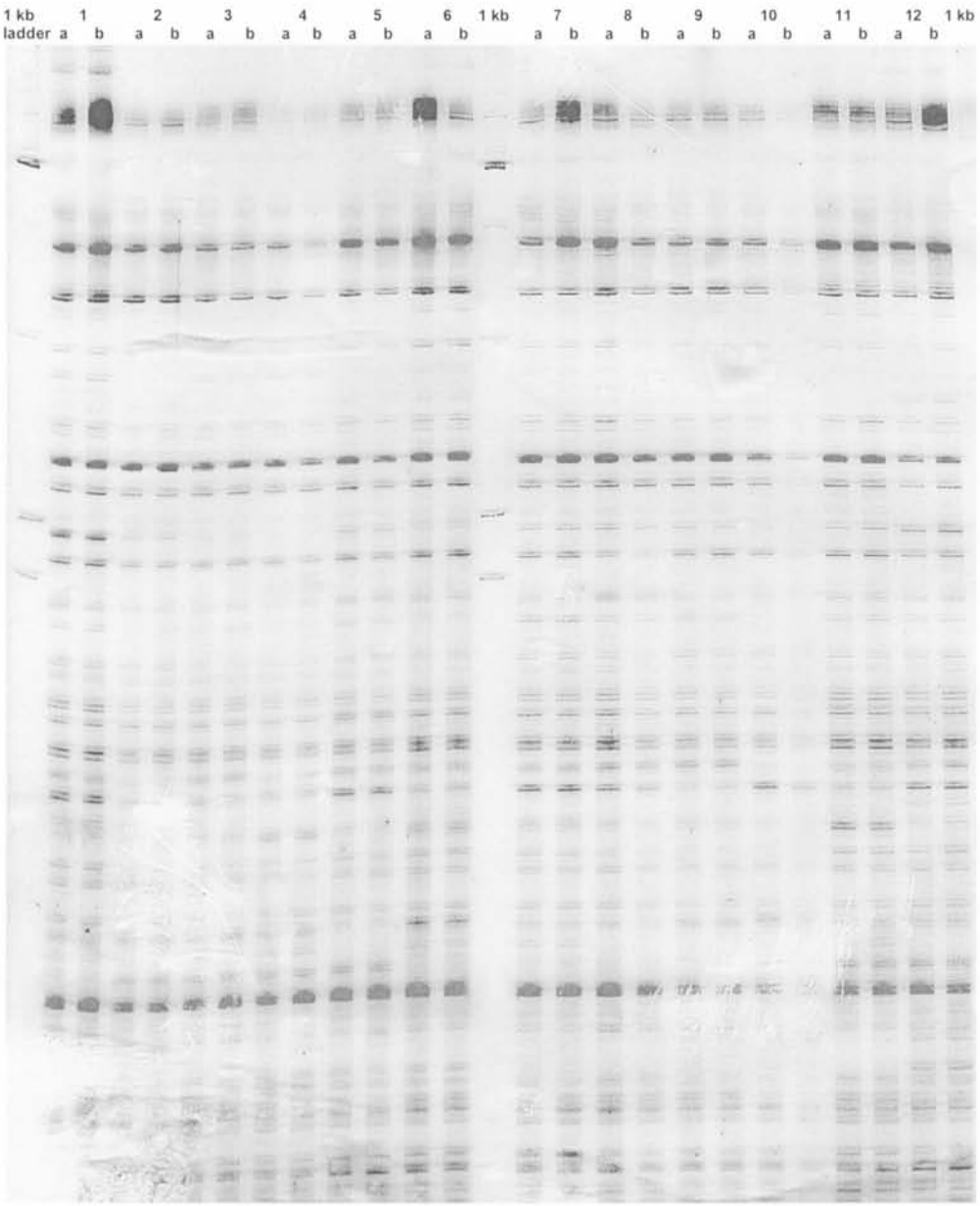


Fig. 4 AFLP fingerprints of genomic DNA of 12 *Ozothamnus* specimens from the Cass population using the primer combination PstI+AC/MseI+CA. Each of the 12 specimens is represented by 2 samples (a and b) derived from 2 separate extractions carried through the complete AFLP process.

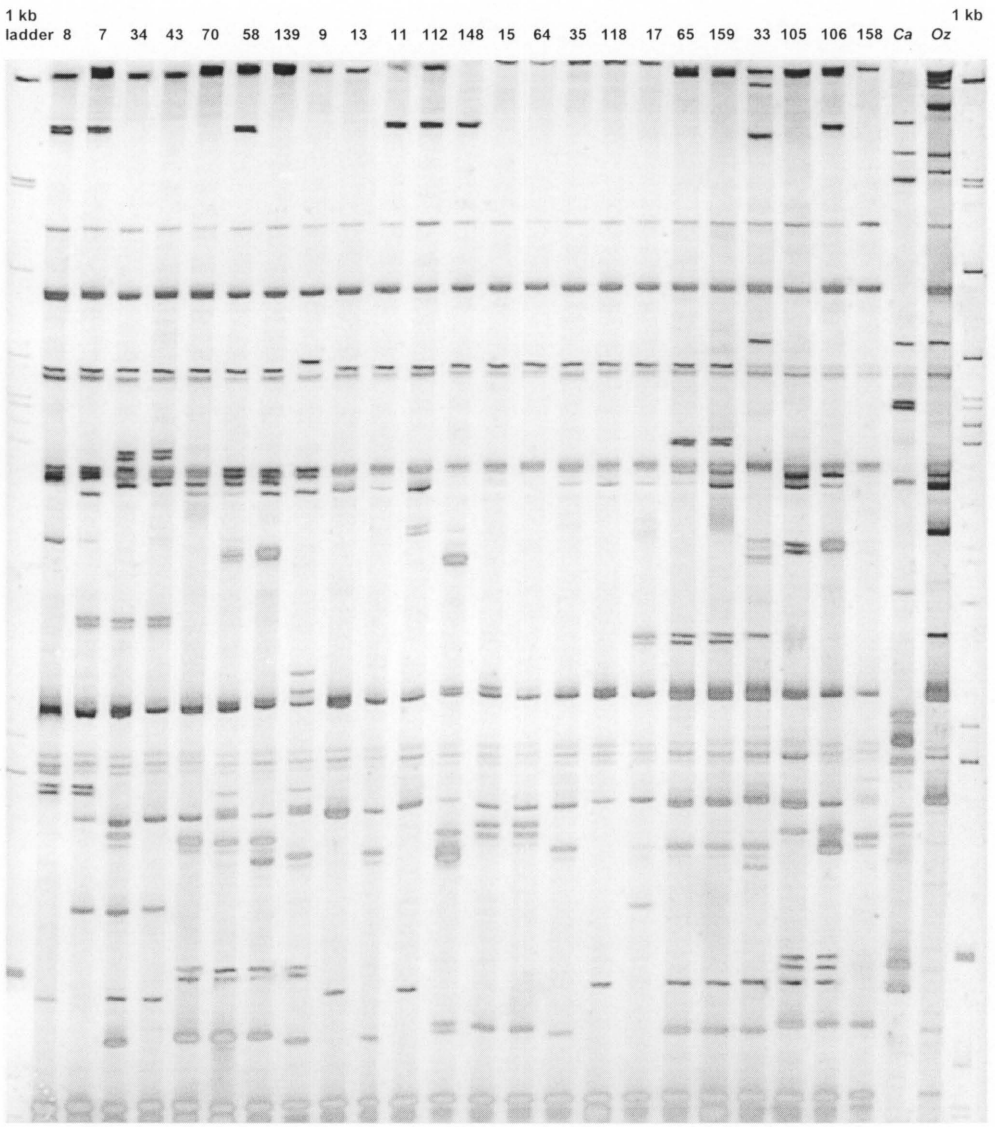


Fig. 5 AFLP fingerprints of genomic DNA of 25 representatives of distinct *Ozothamnus leptophyllus* populations, *Cassinia aculeata* (*Ca*) and *Ozothamnus rodwayi* (*Oz*) using the primer combination PstI+ACT/MseI+CAT.

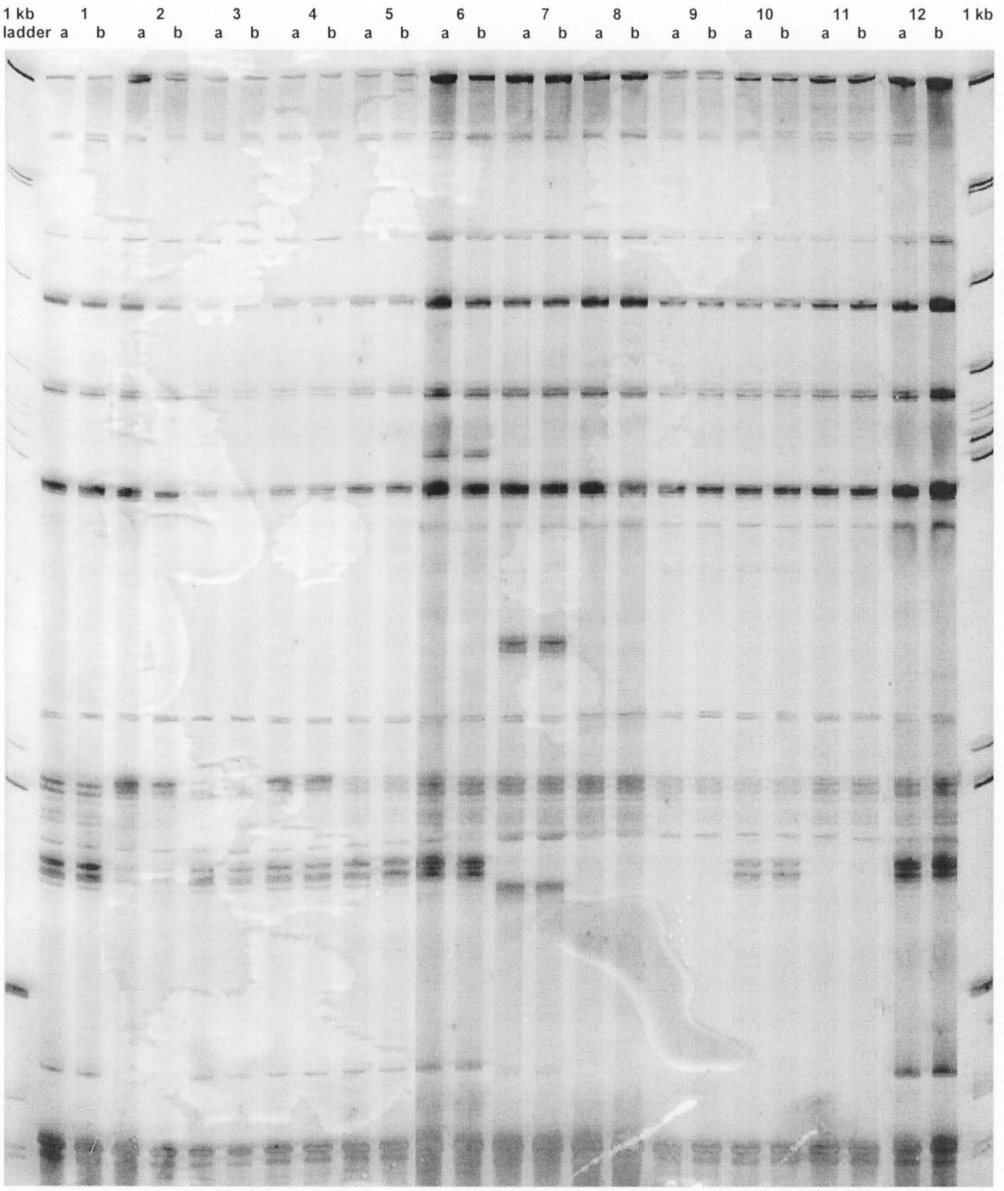


Fig. 6 AFLP fingerprints of genomic DNA of 12 *Ozothamnus* specimens from the Cass population using the primer combination PstI+ACT/MseI+CAT. Each of the 12 specimens is represented by 2 samples (a and b) derived from 2 separate extractions carried through the complete AFLP process.

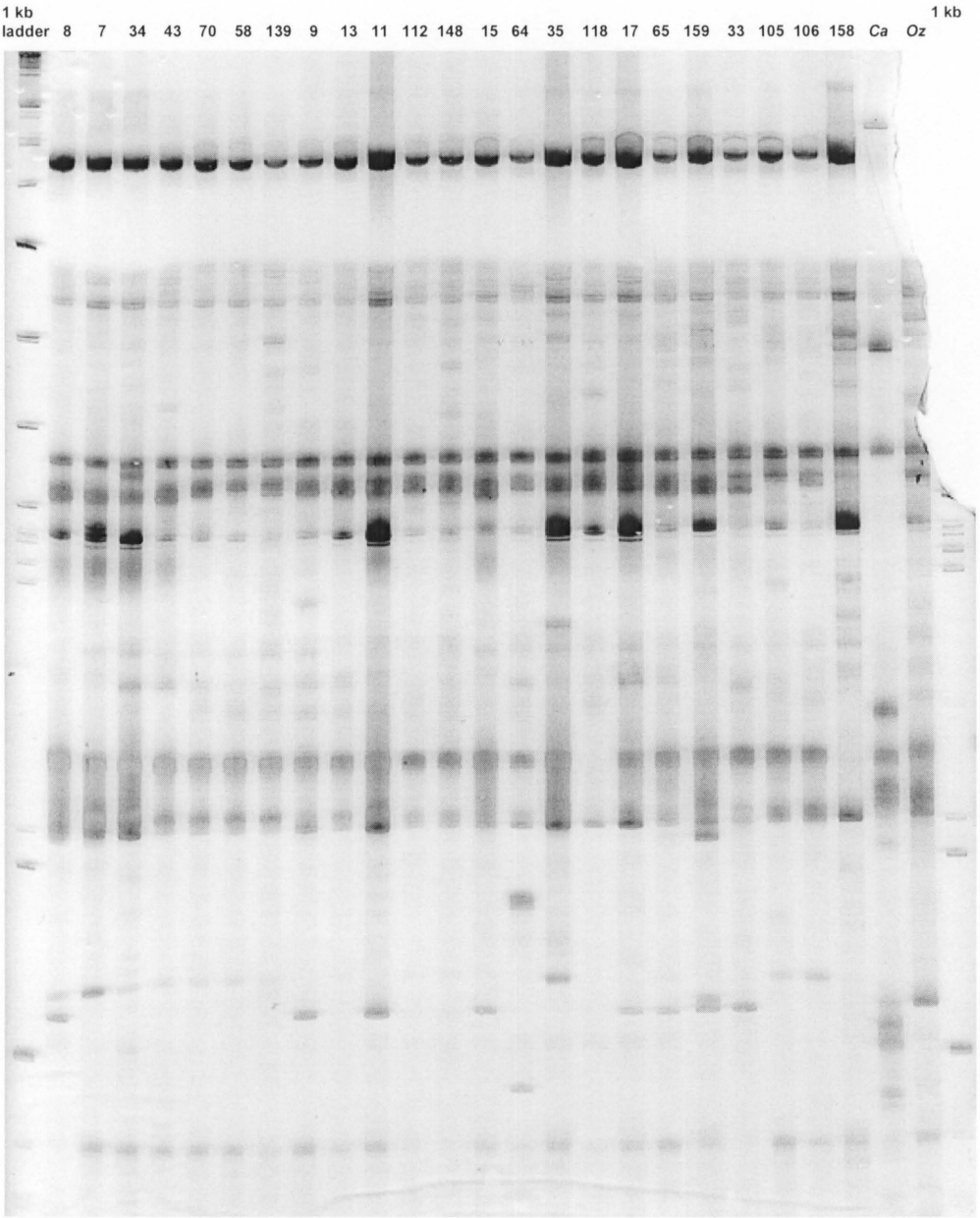


Fig. 7 AFLP fingerprints of genomic DNA of 25 representatives of distinct *Ozothamnus leptophyllus* populations, *Cassinia aculeata* (*Ca*) and *Ozothamnus rodwayi* (*Oz*) using the primer combination PstI+ACG/MseI+CAC.

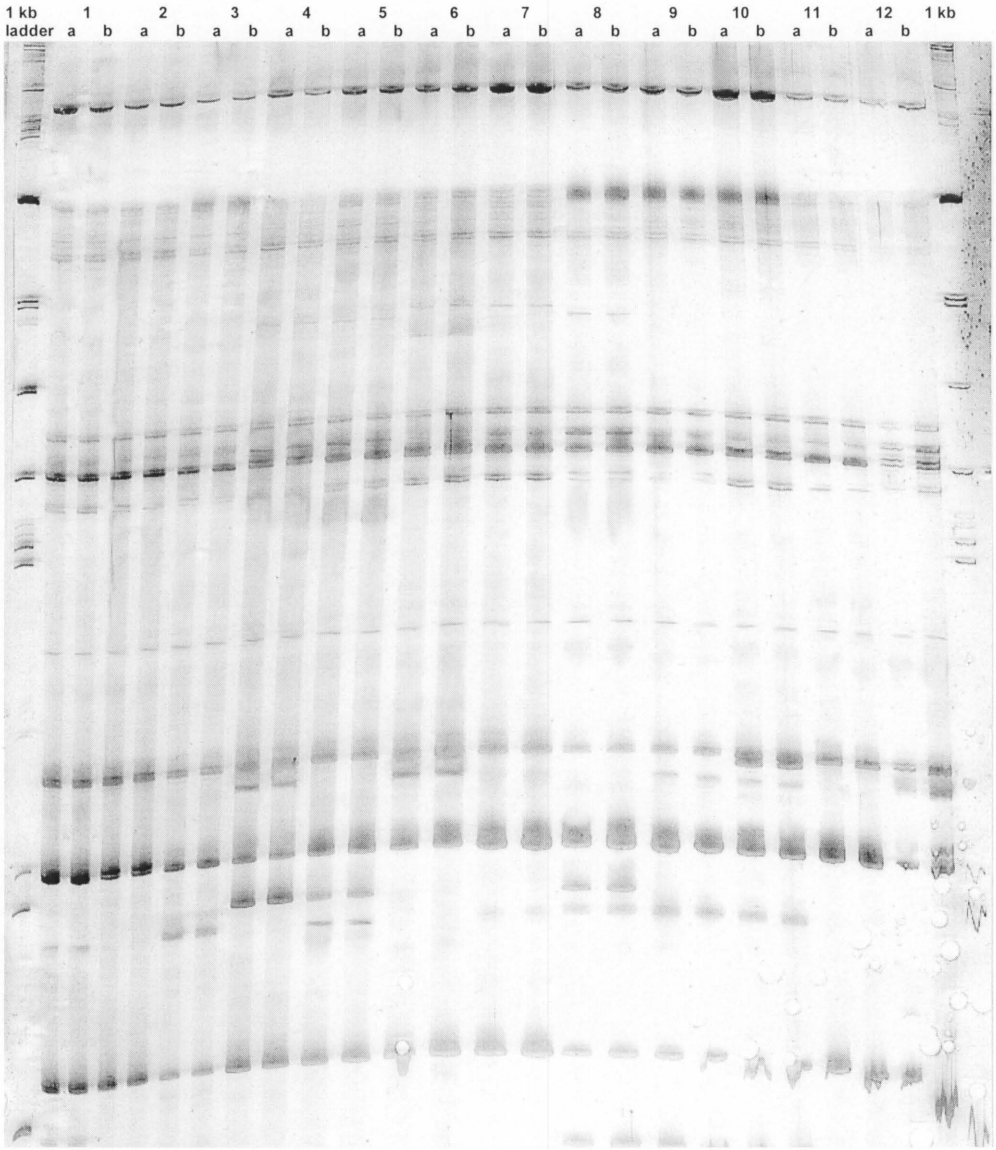


Fig. 8 AFLP fingerprints of genomic DNA of 12 *Ozothamnus* specimens from the Cass population using the primer combination PstI+ACG/MseI+CAC. Each of the 12 specimens is represented by 2 samples (a and b) derived from 2 separate extractions carried through the complete AFLP process.

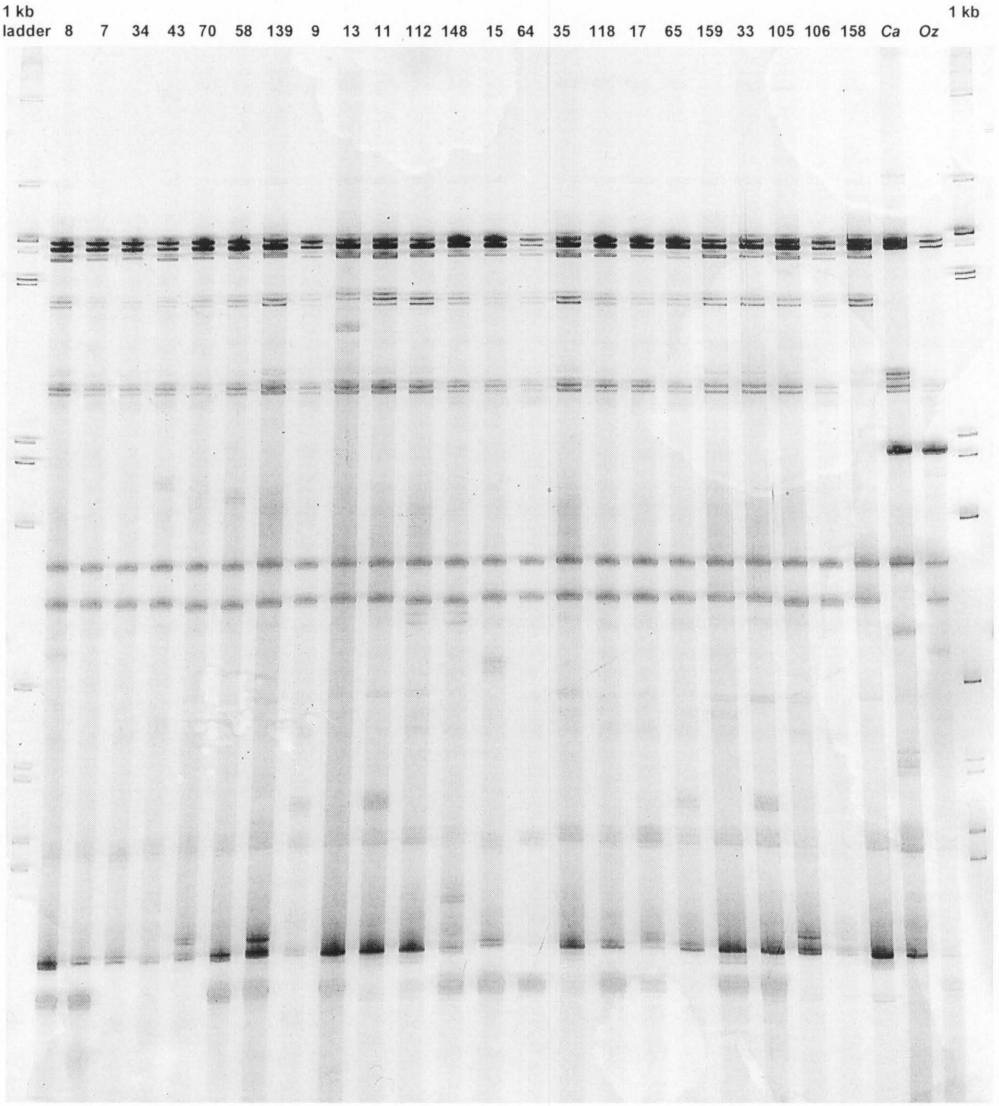


Fig. 9 AFLP fingerprints of genomic DNA of 25 representatives of distinct *Ozothamnus leptophyllus* populations, *Cassinia aculeata* (*Ca*) and *Ozothamnus rodwayi* (*Oz*) using the primer combination PstI+ACA/MseI+CAA.

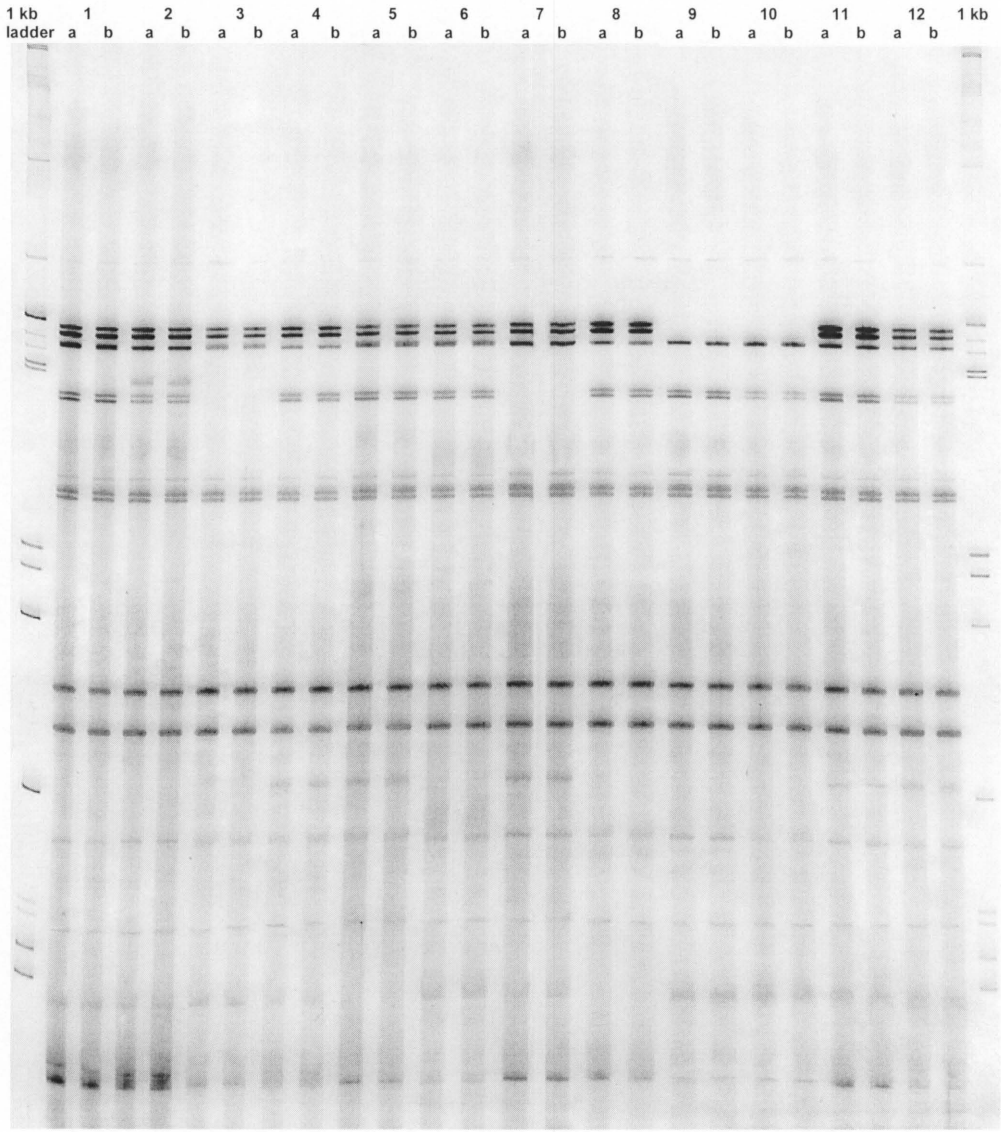


Fig. 10 AFLP fingerprints of genomic DNA of 12 *Ozothamnus* specimens from the Cass population using the primer combination PstI+ACA/MseI+CAA. Each of the 12 specimens is represented by 2 samples (a and b) derived from 2 separate extractions carried through the complete AFLP process.

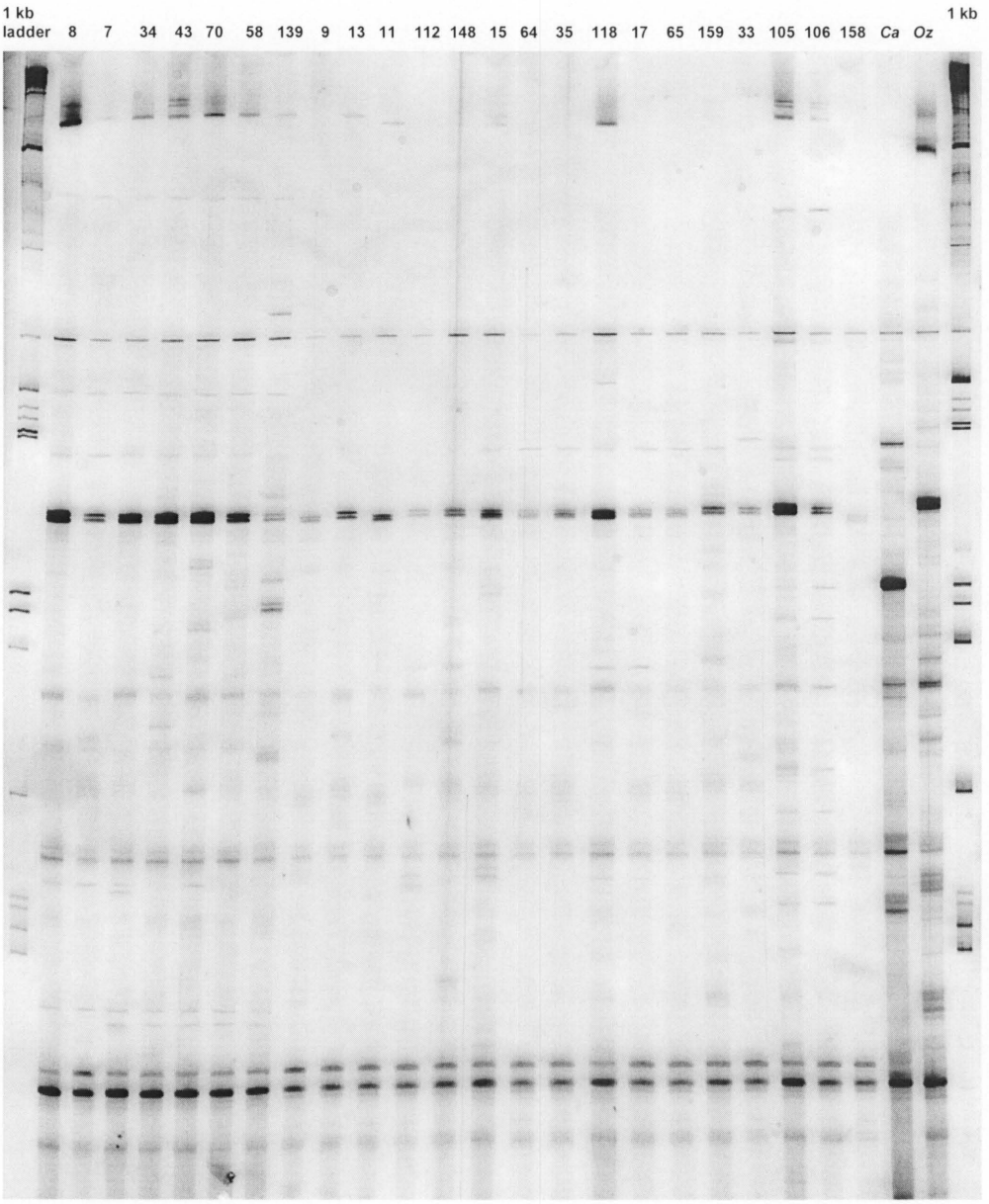


Fig. 11 AFLP fingerprints of genomic DNA of 25 representatives of distinct *Ozothamnus leptophyllus* populations, *Cassinia aculeata* (*Ca*) and *Ozothamnus rodwayi* (*Oz*) using the primer combination PstI+ACG/MseI+CAG.

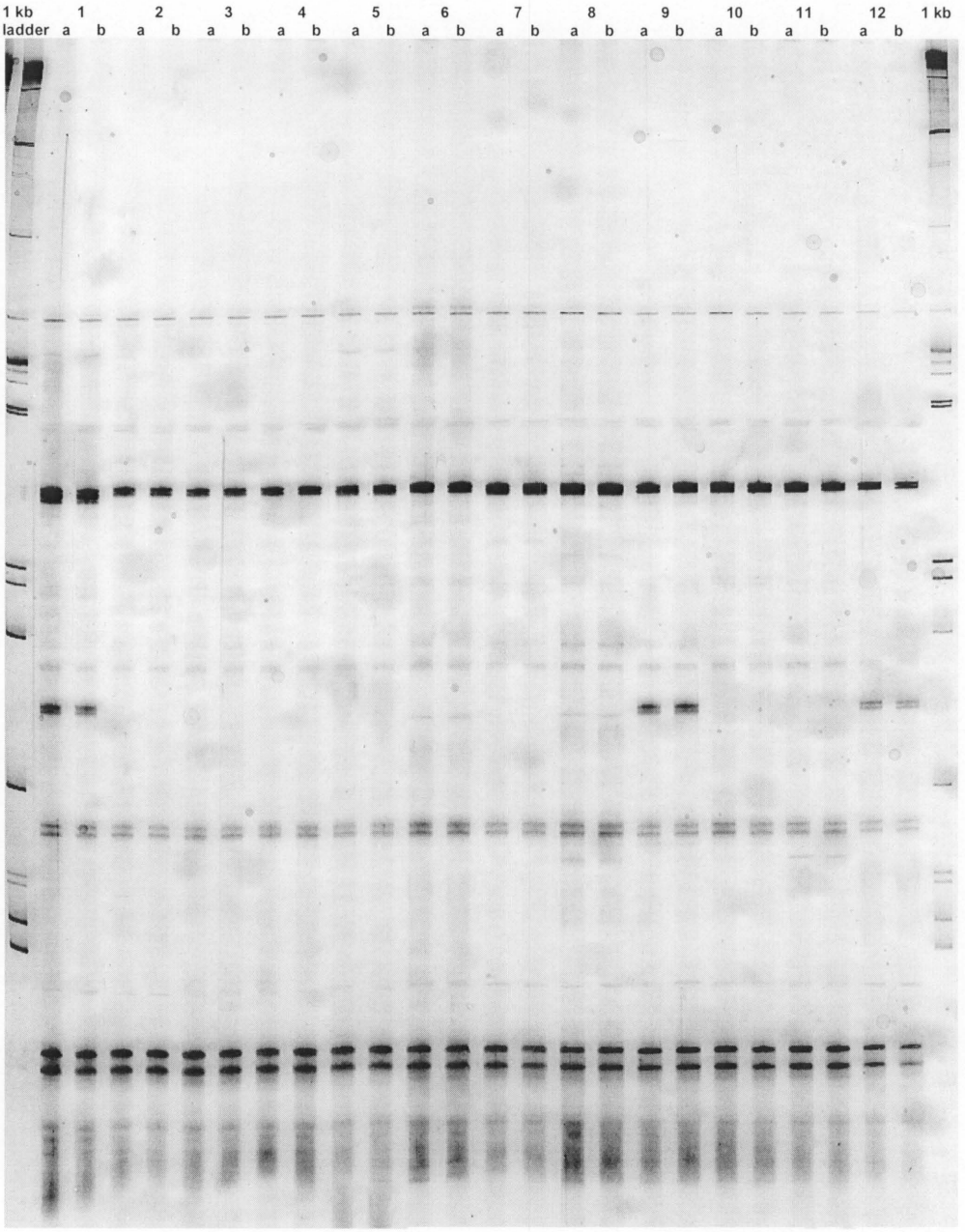


Fig. 12 AFLP fingerprints of genomic DNA of 12 *Ozothamnus* specimens from the Cass population using the primer combination PstI+ACG/MseI+CAG. Each of the 12 specimens is represented by 2 samples (a and b) derived from 2 separate extractions carried through the complete AFLP process.

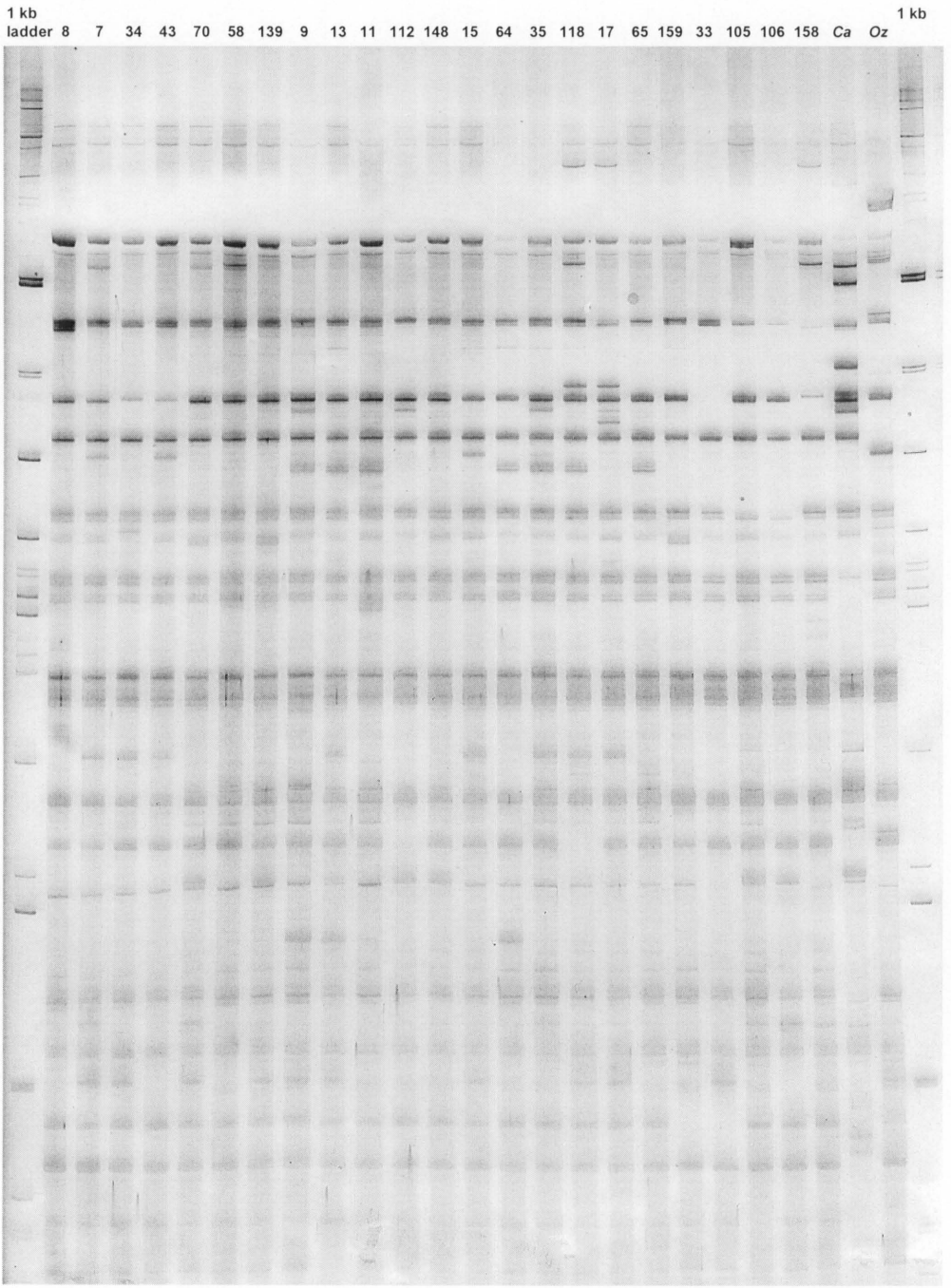


Fig. 13 AFLP fingerprints of genomic DNA of 25 representatives of distinct *Ozothamnus leptophyllus* populations, *Cassinia aculeata* (*Ca*) and *Ozothamnus rodwayi* (*Oz*) using the primer combination PstI+AC/MseI+CAG.

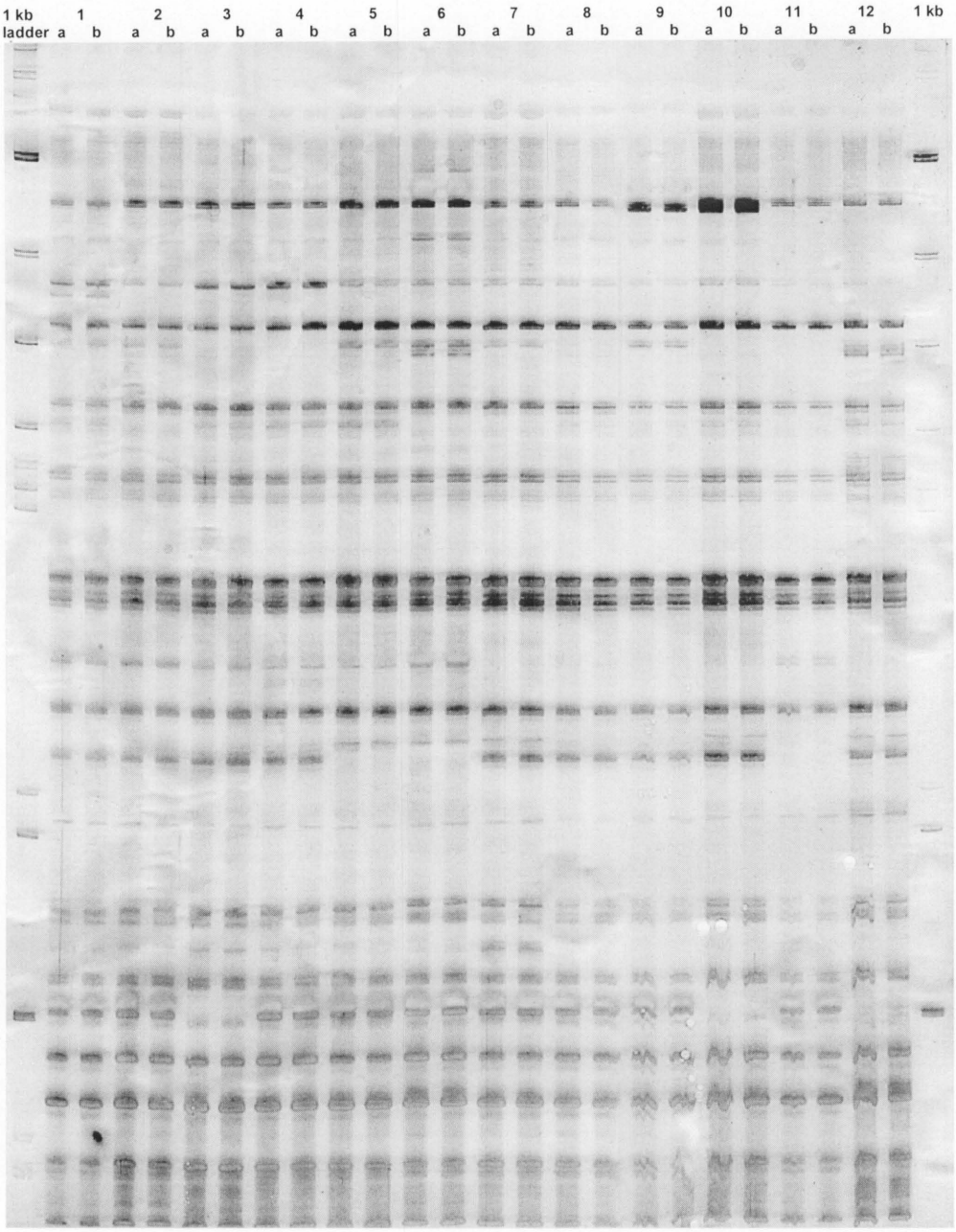


Fig. 14 AFLP fingerprints of genomic DNA of 12 *Ozothamnus* specimens from the Cass population using the primer combination PstI+AC/MseI+CAG. Each of the 12 specimens is represented by 2 samples (a and b) derived from 2 separate extractions carried through the complete AFLP process.

Appendix 13

Molecular Glossary

A - Adenine: A white crystalline purine base. A constituent of DNA and RNA and nucleotides such as ADP and ATP.

ACRYLAMIDE GELS - See POLYACRYLAMIDE GELS.

ADAPTOR - A synthetic double-stranded oligonucleotide that has a blunt end, while the other end has a nucleotide extension that can base pair with a cohesive end created by cleavage of a DNA molecule with a specific restriction endonuclease. The blunt end of the adaptor can be ligated to the ends of a target DNA molecule and the construct can be cloned into a vector by using the cohesive ends of the adaptor.

AFLP - See AMPLIFIED FRAGMENT LENGTH POLYMORPHISM.

AGAR - Malay, agar-agar: A polysaccharide solidifying agent used in nutrient media preparations and obtained from certain types of red algae (Rhodophyta). Both the type of agar and its concentration can affect the growth and appearance of cultured explants.

AGAROSE - The main constituent of agar.

AGAROSE GEL ELECTROPHORESIS - A method for separating nucleic acids (DNA or RNA) within a gel made of agarose in a suitable buffer under the influence of an electrical field. Suitable for separation of large fragments of nucleic acid, separation is based primarily upon the size of the nucleic acid.

ALLELES - Alternative forms of a genetic locus; a single allele for each locus is inherited separately from each parent (e.g., at a locus for eye color the allele might result in blue or brown eyes).

AMPLIFICATION - Multiplication of a piece of DNA in a test-tube into many thousands of millions of copies. The most commonly used process is the polymerase chain reaction (PCR) system.

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) - A type of DNA marker, generated by digestion of genomic DNA with two restriction enzymes to create many DNA fragments, ligation of specific sequences of DNA (called adaptors) to the ends of these fragments, amplification of the fragments via PCR (using a set of primers with sequences corresponding to the adapters, plus various random combinations of three additional bases at the end), and visualization of fragments via gel electrophoresis. The PCR will amplify any fragment whose sequence happens to start with any of the three-base sequences in the set of primers. AFLPs have the important advantage that many markers can be generated with relatively little effort. They are a very useful means of quantifying the extent of genetic diversity within and between populations. Their major disadvantage is that they are not specific to a particular locus and, because they are scored as the presence or absence of a band, heterozygotes cannot be distinguished from homozygotes, i.e., they are inherited in a dominant fashion.

AMPLIFY - To increase the number of copies of a DNA sequence, either in vivo by inserting into a cloning vector that replicates within a host cell, or in vitro by polymerase chain reaction (PCR).

ANNEAL - The pairing of complementary DNA or RNA sequences, via hydrogen bonding, to form a double-stranded polynucleotide. Most often used to describe the binding of a short primer or probe. annealing The process of heating (de-naturing step) and slowly cooling (re-naturing step) double-stranded DNA to allow the formation of hybrid DNA or complementary strands of DNA or of DNA and RNA.

AUTORADIOGRAPHY - A process to detect radioactively labeled molecules (which usually have been separated in a polyacrylamid gel or agarose gel) based on their ability to create an image on photographic or X-ray film. This process does not result in a linear relationship between the intensity of the signal and the amount of radioactivity unless special steps are taken. There is now increasing use of phosphorimagers

and other modern devices to detect and quantitate radioactive molecules which have been separated in gels.

BAND - See RESTRICTION FRAGMENT

BASE - A cyclic, nitrogen-containing compound that is one of the essential components of nucleic acids. Exists in five main forms (adenine, A; guanine, G; thymine, T; cytosine, C; uracil, U). A and G have a similar structure and are called purines; T, C and U have a similar structure and are called pyrimidines. A base joined to a ribose sugar joined to a phosphate group is a nucleotide - the building block of nucleic acids.

BASE PAIR (bp) - The two strands that constitute DNA are held together by specific hydrogen bonding between purines and pyrimidines (A pairs with T; and G pairs with C). The size of a nucleic acid molecule is often described in terms of the number of base pairs (symbol: bp) or thousand base pairs (kilobase pairs; symbol: kb; a more convenient unit) it contains.

BASE SEQUENCE - the order of nucleotide bases in a DNA molecule (see NUCLEOTIDE SEQUENCE)

bp - Abbreviation for base pair.

BLUNT END - A terminus of a duplex DNA molecule which ends precisely at a base pair, with no overhang (unpaired nucleotide) in either strand. Some but not all restriction endonucleases leave blunt ends after cleaving DNA. Blunt-ended DNA can be ligated nonspecifically to other blunt-ended DNA molecules (compare with STICKY END).

BUFFER - A solution that resists change in pH when an acid or alkali is added, or when solutions are diluted.

C - Cytosine: A purine derivative that is one of the major component bases of nucleotides and the nucleic acids, DNA and RNA. A nitrogenous base, one member of the base pair G- C (guanine and cytosine)

CENTRIFUGATION - Separating molecules by size or density using centrifugal forces generated by a spinning rotor. G-forces of several hundred thousand times gravity are generated in ultracentrifugation.

CENTRIFUGE - A device in which solid or liquid particles of different densities are separated by rotating them in a tube in a horizontal circle. The denser particles tend to move along the length of the tube to a greater radius of rotation, displacing the lighter particles to the other end.

CLONING - In recombinant DNA technology, the use of DNA manipulation procedures to produce multiple copies of a single gene or segment of DNA is referred to as cloning DNA.

CUT - Slang: to make a double-stranded break in DNA, usually with a restriction endonuclease. E.g., "The DNA was cut with EcoRI and run out on a 1% agarose gel."

CTAB - cetyltrimethylammonium bromide.

DEIONIZED WATER - Water which is free of most inorganic (not completely free, since Na is present in ample quantities) and most organic compounds.

DENATURATED DNA - Duplex DNA that has been converted to single strands by breaking the hydrogen bonds of complementary nucleotide pairs. Usually achieved by heating.

DENATURATION - With respect to nucleic acids, refers to the conversion from double-stranded to the single-stranded state, often achieved by heating or alkaline conditions. This is also called "melting" DNA. Withrespect to proteins, refers to the disruption of tertiary and secondary structure, often achieved by heat, detergents, chaotropes, and sulfhydryl-reducing agents.

DENATURING GEL - An agarose or acrylamide gel run under conditions which destroy secondary or tertiary protein or RNA structure. For protein, this usually means the inclusion of 2-ME (which reduces

disulfide bonds between cysteine residues) and SDS and/or urea in an acrylamide gel. For RNA, this usually means the inclusion of formaldehyde or glyoxal to destroy higher ordered RNA structures. In DNA sequencing gels, urea is included to denature dsDNA to ssDNA strands. In denaturing gels, macromolecules tend to be separated on the basis of size and (to some extent) charge, while shape and oligomerization of molecules are not important.

DETERGENT - Substance which lowers the surface tension of a solution, improving its cleaning properties.

DIGEST - To cut DNA molecules with one or more restriction endonucleases.

DNA - Deoxyribonucleic acid; formerly spelt desoxyribonucleic acid: The long chain of molecules in most cells that carries the genetic message and controls all cellular functions in most forms of life. The information-carrying genetic material that comprises the genes. DNA is a macro-molecule composed of a long chain of deoxyribonucleotides joined by phospho-diester linkages. Each deoxyribonucleotide contains a phosphate group, the five-carbon sugar 2-deoxribose, and a nitrogen-containing base. The genetic material of most organisms and organelles so far examined is double-stranded DNA; a number of viral genomes consist of single-stranded DNA or single-or double-stranded RNA. In double-stranded DNA, the two strands run in opposite (anti-parallel) directions and are coiled round one another in a double helix. Purine bases on one strand specifically hydrogen bond with pyrimidine bases on the other strand, according to the Watson-Crick rules (A pairs with T; G pairs with C). Hence a constant width for the double helix of 20 Å (2.0 nm) is maintained. In the B-form, DNA adopts a right-handed helical conformation, with each chain making a complete turn every 34 Å (3.4 nm), or once every ten bases.

DNA FINGERPRINT - The unique pattern of DNA fragments identified originally by Southern hybridization (using a probe that binds to a polymorphic region of DNA) or now by polymerase chain reaction (PCR) (using primers flanking the polymorphic region).

DNA LIGASE - An enzyme (usually from the T4 bacteriophage) which catalyzes formation of a phosphodiester bond between two adjacent bases from double-stranded DNA fragments. RNA ligases also exist, but are rarely used in molecular biology.

DNA SEQUENCE - The relative order of base pairs, whether in a fragment of DNA, a gene, a chromosome, or an entire genome.

DNA SEQUENCING - Procedures for determining the nucleotide sequence of a DNA fragment. There are two common methods for doing this: the Maxam and Gilbert technique (chemical degradation), that uses different chemicals to break the DNA into fragments at specific bases; or the Sanger technique (called the di-deoxy or chain-terminating method) uses DNA polymerase to make new DNA chains, with di-deoxy nucleotides (chain terminators) to stop the chain randomly as it grows. In both cases, the DNA fragments are separated according to length by polyacrylamide gel electrophoresis, enabling the sequence to be read directly from the gel.

dNTP - A deoxyribonucleotide (A,G, C, or T). Free dNTPs in excess are an essential component of PCR.

DOMINANT - Describing an allele whose effect with respect to a particular trait is the same in heterozygotes as in homozygotes. The opposite is recessive.

EDTA - Ethylene-diaminetetraacetic acid.

ELECTROPHORESIS - See GEL ELECTROPHORESIS

ENZYME - A protein that acts as a catalyst, speeding the rate at which a biochemical reaction proceeds but not altering the direction or nature of the reaction.

ETHIDIUM BROMIDE - Intercalates within the structure of nucleic acids in such a way that they fluoresce under UV light. Ethidium bromide staining is commonly used to visualize RNA or DNA in agarose gels placed on Ultra violet light boxes. Proper precautions are required, because the ethidium bromide is highly mutagenic and the UV light damaging to the eyes. Ethidium bromide is also included in cesium

chloride gradients during ultracentrifugation, to separate supercoiled circular DNA from linear and relaxed circular DNA.

EPPENDORF TUBES - cf. Cryotube. Small (? 1.5 ml) flip-top plastic vial for storing reagents, samples etc. Less expensive than cryotubes but also more subject to leakage.

EXTENSION - See OVERHANG

FINGERPRINTING - See DNA FINGERPRINTING

FRAGMENT - See RESTRICTION FRAGMENT.

G - Guanine: A purine derivative that is one of the major component bases of nucleotides and the nucleic acids, DNA and RNA. A nitrogenous base, one member of the base pair G- C (guanine and cytosine)

GEL - A lyophilic colloid that has coagulated to a rigid or jelly-like solid. It is used for the electrophoretic separation of nucleic acids or proteins, and for encapsulation. See GEL ELECTROPHORESIS.

GEL ELECTROPHORESIS - An analytical method for separating molecules according to their size. Samples are put at one end of a slab of polymer gel; an electric field across the gel pulls the molecules through it; the smaller molecules pass more easily and so move towards the other end faster; the various sizes of molecules end up at different positions according their size. Gels are made from different materials, but common combinations are: Various chemicals can be included in the gel to help separation, such as the detergent sodium dodecyl sulphate (SDS) in protein gels to unfold proteins, or urea in DNA sequencing gels, which unfolds DNA. See POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE).

GENOME - The complete set of genetic information defining a particular animal, plant, organism or virus.

GENOMIC LIBRARY - A DNA library which contains DNA fragments hopefully representing each region of the genome of an organism, virus, etc, cloned into individual vector molecules for subsequent selection and amplification. The DNA fragments are usually very small in size compared with the genome. Such libraries are cDNA libraries when prepared from RNA viruses.

GENOTYPE - The genetic constitution of an organism; determined by its nucleic acid sequence. As applied to viruses, the term implies a group of evolutionarily related viruses possessing a defined degree of nucleotide sequence relatedness.

HOMOLOGY - Indicates similarity between two different nucleotide or amino acid sequences, often with potential evolutionary significance. It is probably better to use more quantitative and descriptive terms such as nucleotide "identity" or, in the case of proteins, amino acid "identity" or "relatedness" (the latter refers to the presence of amino acids residues with similar polarity/charge characteristics at the same position within a protein).

kb - See BASE PAIR; KILOBASE.

KILOBASE (kb) - A length unit equal to 1 000 base pairs of a double-stranded nucleic acid molecule. One kilobase of double-stranded DNA has a mass of about 660 kilodalton.

LABELLING - The process of replacing a stable atom in a compound with a radioactive isotope of the same element to enable it to be detected by autoradiography or other techniques. Increasingly, radioactive labelling is being replaced by fluorescent labelling. The method is used to trace the path of the labelled compound through a biological or chemical system.

LADDER - A series of known-size fragments run in a gel to allow sizing of fragments of target DNA run in other lanes. One commonly used ladder is phagelambda cut with Pst [yields fragments of 216, 211, 200, 164 and 150 bp].

LIGASE - See DNA LIGASE.

LIGATE (to) - The process of joining two or more DNA fragments.

LIGATION - See DNA LIGASE.

MAPPING - Determining the location of a locus (gene or genetic marker) on a chromosome.

MARKER - An identifiable DNA sequence that facilitates the study of inheritance of a trait or a gene. Such markers are used in mapping the order of genes along chromosomes and in following the inheritance of particular genes: genes closely linked to the marker will generally be inherited with it. Markers must be readily identifiable in the phenotype, for instance by controlling an easily observable feature (such as eye colour) or by being readily detectable by molecular means, e.g., microsatellite markers.

MICROSATELLITE - A segment of DNA characterized by the occurrence of a variable number of copies (from a few up to 30 or so) of a sequence of around 5 or fewer bases (called a repeat unit, q.v.). A typical microsatellite is the repeat unit AC, which occurs at approximately 100 000 different sites in a typical mammalian genome. At any one site (locus), there are usually several different "alleles," each identifiable according to the number of repeat units. These alleles can be detected by PCR (q.v.), using primers designed from the unique sequence that is located on either side of the microsatellite. When the PCR product is run on an electrophoretic gel, alleles are seen to differ in length in units equal to the size of the repeat unit, e.g., if the primers correspond to the unique sequence immediately on either side of the microsatellite and are each 20 bases long, and an individual is heterozygous for an AC microsatellite with one allele comprising 5 repeats and the other comprising 6 repeats, the heterozygote will exhibit two bands on the gel, one band being 20 + (2 ′ 5) + 20 = 50 bases long, and the other allele being 20 + (2 ′ 6) + 20 = 52 bases long. Microsatellites have been the standard DNA marker: they are easily detectable by PCR, and they tend to be evenly located throughout the genome. Thousands have been mapped in many different species.

M – Mole: Amount of substance that has a weight in grams numerically equal to the molecular weight of the substance. Also called gram molecular weight. A mole contains 6.023 ′ 1023 molecules or atoms of a substance.

NUCLEOTIDE - A subunit of DNA or RNA consisting of a nitrogenous base (adenine, guanine, thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA), a phosphate molecule, and a sugar molecule (deoxyribose in DNA and ribose in RNA). Thousands of nucleotides are linked to form a DNA or RNA molecule. See DNA, BASE PAIR, RNA.

NUCLEOTIDE SEQUENCE - The order of nucleotide bases in a DNA molecule.

OLIGONUCLEOTIDE - A short molecule (usually 6 to 100 nucleotides) of single-stranded DNA.

OVERHANG - A terminus of a duplex DNA molecule which has one or more unpaired nucleotides in one of the two strands (hence either a 3' or 5' overhang). Cleavage of DNA with many restriction endonucleases leaves such overhangs.

PCR - See POLYMERASE CHAIN REACTION.

pH - A measure of acidity and alkalinity. Equal to the log of the reciprocal of the hydrogen ion concentration of a solution, expressed in grams per litre. A reading of 7 is neutral (e.g., pure water), whereas below 7 is acid and above 7 is alkaline.

PHENOTYPE - (Gr. phaneros, showing + type) The appearance of other characteristics of an organism resulting from the interaction of its genetic constitution with the environment.

PHYLOGENY - A diagram illustrating the deduced evolutionary history of populations of related organisms.

POLYACRYLAMIDE GEL - Used to separate proteins and smaller DNA fragments and oligonucleotides by electrophoresis. When run under conditions which denature proteins (i.e., in the presence of 2-mercaptoethanol, SDS, and possibly urea), molecules are separated primarily on the basis of size.

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) - A method for separating nucleic acid or protein molecules according to their molecular size. The molecules migrate through the inert gel matrix under the influence of an electric field. In the case of nucleic acids, denaturing agents such as formamide, urea or methyl mercuric hydroxide are often incorporated into the gel itself, which may also be run at high temperature. PAGE is used to separate the products of DNA-sequencing reactions and the gels employed are highly denaturing, since molecules differing in size by a single nucleotide must be resolved.

POLYMERASE - An enzyme which catalyses the addition of a nucleotide to a nucleic acid molecule. There are a wide variety of RNA and DNA polymerases which have a wide range of specific activities and which operate optimally under different conditions. In general, all polymerases require templates upon which to build a new strand of DNA or RNA; however, DNA polymerases also require a primer to initiate the new strand, while RNA polymerases start synthesis at a specific promoter sequence.

POLYMERASE CHAIN REACTION (PCR) - A procedure that amplifies a particular DNA sequence. It involves multiple cycles of denaturation, annealing to oligonucleotide primers, and extension (polynucleotide synthesis), using a thermostable DNA polymerase (see Taq POLYMERASE), deoxyribonucleotides, and primer sequences in multiple cycles of denaturation-renaturation-DNA synthesis. See POLYMERASE.

POLYMERISATION - a chemical reaction in which one or more small molecules combine to form larger molecules.

POLYMORPHISM - Variation within a DNA or RNA sequence.

POPULATION - A local group of organisms belonging to the same species and interbreeding.

PRIMER - An oligonucleotide which is complementary to a specific region within a DNA or RNA molecule, and which is used to prime (initiate) synthesis of a new strand of complementary DNA at that specific site, in a reaction or series of reactions catalyzed by a DNA polymerase. The newly synthesized DNA strand will contain the primer at its 5' end. Typically, primers are chemically synthesized oligonucleotides 15-50 nucleotides in length, selected on the basis of a known sequence. However, "random primers" (shorter oligonucleotides, about 6 nucleotides in length, and comprising all possible sequences) may be used to prime DNA synthesis from DNA or RNA of unknown sequence.completely known, but probably serves to enhance stability of the RNA. Is frequently used to select mRNA for cloning purposes by annealing to a column containing a matrix bound to poly-uridylic acid.

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD; pronounced 'rapid') - A technique using single, short (usually 10-mer) synthetic oligonucleotide primers for PCR. The primer, whose sequence has been chosen at random, initiates replication at its complementary sites on the DNA, producing fragments up to about 2 kb long, which can be separated by electrophoresis and stained with ethidium bromide. A primer can exhibit polymorphism between individuals, and polymorphic fragments can be used as markers.

RAPD - See RANDOM AMPLIFIED POLYMORPHIC DNA.

RARE- CUTTER ENZYME - See RESTRICTION ENZYME CUTTING SITE.

RECOGNITION SEQUENCE - See RECOGNITION SITE.

RECOGNITION SITE - A nucleotide sequence – composed typically of 4, 6 or 8 nucleotides – that is recognized by and to which a restriction endonuclease (restriction enzyme) binds. For type II restriction enzymes (those used in gene-cloning experiments) it is also the sequence within which the enzyme specifically cuts (and their corresponding enzymes methylate) the DNA, i.e., for type II enzymes, the recognition site and the target site are the same sequence. Type I enzymes bind to their recognition site and then cleave the DNA at some more or less random position outside that recognition site. cf restriction site.

rDNA - ribosomal DNA; the genes for several classes of ribosomal RNA molecules that go into the construction of ribosomes, usually in long tandem arrays in the chromosomes.

RESTRICTION ENDONUCLEASE - A bacterial enzyme which recognizes a specific palindromic sequence (recognition sequence) within a double-stranded DNA molecule and then catalyzes the cleavage of both strands at that site. Also called a restriction enzyme. Restriction endonucleases may generate either blunt or sticky ends at the site of cleavage.

RESTRICTION ENZYME - See RESTRICTION ENDONUCLEASE.

RESTRICTION ENZYME CUTTING SITE - A specific nucleotide sequence of DNA at which a particular restriction enzyme cuts the DNA. Some sites occur frequently in DNA (e.g., every several hundred base pairs), others much less frequently (rare- cutter; e.g., every 10,000 base pairs).

RESTRICTION FRAGMENT - A fragment of DNA produced by cleaving (digesting, cutting) a DNA molecule with one or more restriction endonucleases.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) - The occurrence of variation in the length of DNA fragments that are produced after cleavage with a type II restriction endonuclease. The differences in DNA lengths are due to the presence or absence of recognition site(s) for that particular restriction enzyme. RFLPs were initially detected using hybridization with DNA probes after separation of digested genomic DNA by gel electrophoresis (Southern analysis). Now they are typically detected by electrophoresis of digested PCR product.

RESTRICTION FRAGMENTS - DNA fragments generated by digestion of a DNA preparation with one or more restriction endonucleases; usually separated by agarose gel electrophoresis and visualized by ethidium bromide staining under UV light (or alternatively subjected to Southern blot analysis).

RESTRICTION SITE - The specific nucleotide sequence in DNA that is recognized by a type II restriction endonuclease and within which it makes a double-stranded cut. Restriction sites usually comprise four or six base pairs that typically are palindromic (q.v.), e.g., 5' GGCC3' 3' CCGG5' The two strands may be cut either opposite to one another, to create blunt ends, or in a staggered manner, giving sticky ends, depending on the enzyme involved. See RESTRICTION ENDONUCLEASE, RECOGNITION SEQUENCE.

RFLP - See RESTRICTION FRAGMENT LENGTH POLYMORPHISM.

RIBONUCLEIC ACID (RNA) - A chemical found in the nucleus and cytoplasm of cells; it plays an important role in protein synthesis and other chemical activities of the cell. The structure of RNA is similar to that of DNA. There are several classes of RNA molecules, including messenger RNA, transfer RNA, ribosomal RNA, and other small RNAs, each serving a different purpose.

RNA: See RIBONUCLEIC ACID.

SEQUENCE - See BASE SEQUENCE.

SEQUENCING - The determination of the order of nucleotides in a DNA or RNA molecule, or that of amino acids in a polypeptide chain. See DNA SEQUENCING.

SIMPLE SEQUENCE REPEAT (SSR) - See MICROSATELLITE.

SOUTHERN BLOT - DNA is separated by electrophoresis (usually in agarose gels), then transferred to nitrocellulose paper or other suitable solid-phase matrix (e.g., nylon membrane), and denatured into single strands so that it can be hybridised with a specific probe. The Southern blot was developed by E.M. Southern, a molecular biologist in Edinburgh. Northern and western blots were given contrasting names to reflect the different target substances (RNA and proteins, respectively) that are subjected in these procedures to electrophoresis, blotting and subsequent detection with specific probes.

SOUTHERN HYBRIDISATION - A procedure in which a cloned, labelled segment of DNA is hybridised to DNA restriction fragments on a Southern blot (q.v.).

SPACER DNA - The DNA found between two genes. Can be either transcribed or nontranscribed.

ss - Single stranded.

SSR - simple sequence repeat. See MICROSATELLITE.

T - Thymine: A pyrimidine derivative that is one of the major component bases of nucleotides and the nucleic acids, DNA and RNA. A nitrogenous base, one member of the base pair A- T (adenine- thymine). The thymine residue in DNA.

TAE - Tris-acetic acid-EDTA buffer used for electrophoresis of DNA.

Taq POLYMERASE - A DNA polymerase which is very stable at high temperatures, isolated from the thermophilic bacterium Thermus aquaticus. Very useful in PCR reactions which must cycle repetitively through high temperatures during the denaturation step. See POLYMERASE.

TBE - Tris, boric acid, EDTA electrophoresis buffer.

TE - Tris-EDTA buffer used to dilute DNA.

TRANSCRIPTION - The copying of a DNA template into a single-stranded RNA molecule. The processes whereby the transcriptional activity of eukaryotic genes are regulated are complex, involve a variety of accessory transcriptional factors which interact with promoters and polymerases, and constitute one of the most important areas of biological research today.